BRIDGING THE GAP BETWEEN POLICY AND SCIENCE IN ASSESSING THE HEALTH STATUS OF MARINE ECOSYSTEMS

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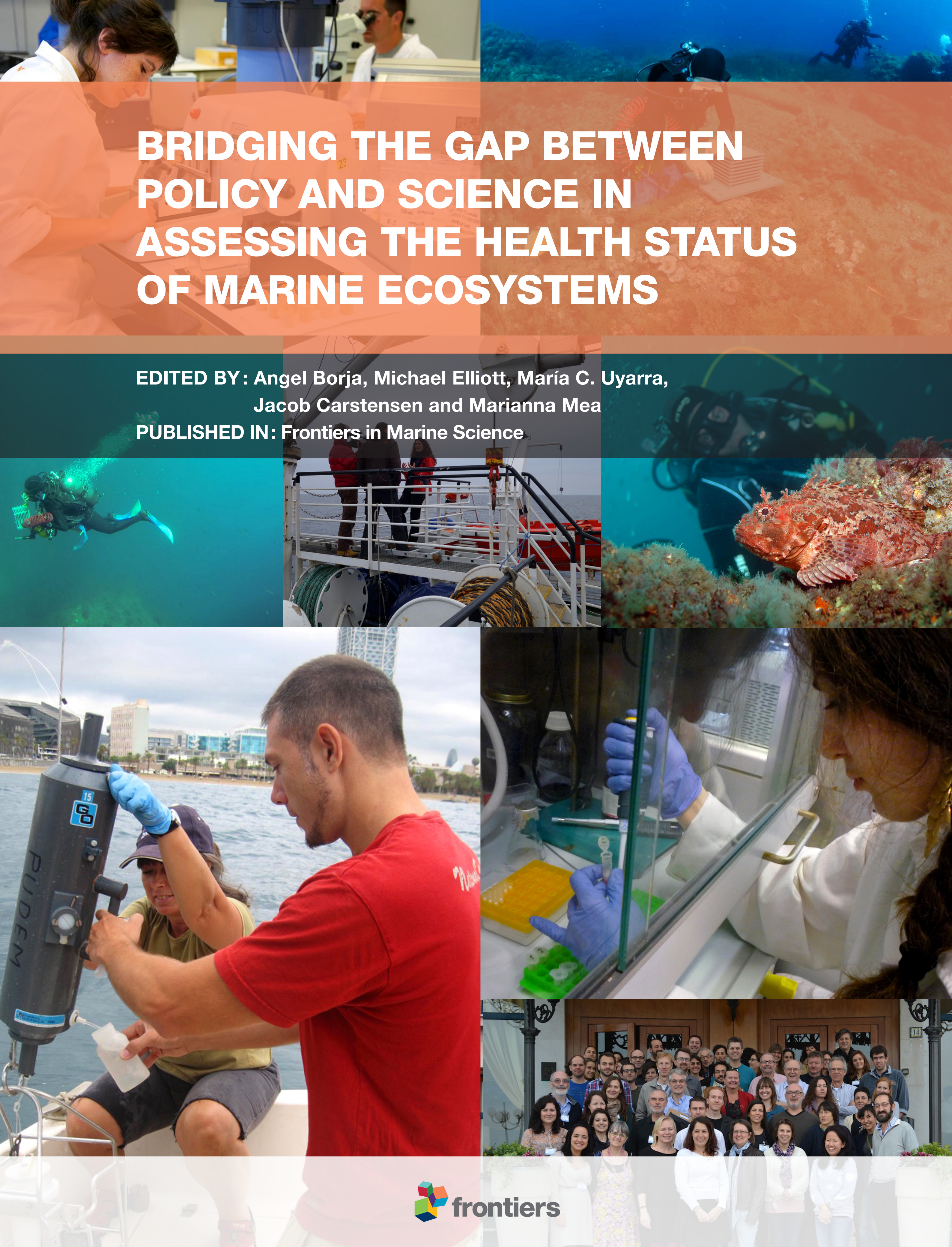

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BRIDGING THE GAP BETWEEN POLICY AND SCIENCE IN ASSESSING THE HEALTH STATUS OF MARINE ECOSYSTEMS

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DEVOTES researchers at work.
Cover photos courtesy of AZTI-Tecnalia, Spain (photos 1, 2 and 5), CNRS, France (photo 3), SYKE, Finland (photo 4), CSIC, Spain (photo 6), CoNISMa, Italy (photo 7) and Ecoreach, Italy (photo 8).

Policy and Science: Finding the Common Ground for Advancing in the Assessment of Marine Ecosystems

Marine management requires approaches which bring together the best research from the natural and social sciences. It requires stakeholders to be well-informed by science and to work across administrative and geographical boundaries, a feature especially important in the inter-connected marine environment. Marine management must ensure that the natural structure and functioning of ecosystems is maintained to provide ecosystem services. Once those marine ecosystem services have been created, they deliver societal goods as long as society inputs its skills, time, money and energy to gather those benefits. However, if societal goods and benefits are to be limitless, society requires appropriate administrative, legal and management mechanisms to ensure that the use of such benefits do not impact on environmental quality, but instead support its sustainable use.

All of this requires for Policy and Science: Finding the Common Ground for Advancing in the Assessment of Marine Ecosystems to find a common ground in which scientists should advance science and provide policy makers with the best available knowledge and interpretation of the functioning of marine ecosystems. Hence, policy makers, recognizing the complexity and vulnerability of this system, could, through informed decisions, establish the framework for the development of sustainable societies. To this end, adequate and fit-for-purpose monitoring is needed. It should be based on quantitative and qualitative indicators to determine both trends in the system and assess whether management actions are successful. Finally, given current economic restrictions, all of this has to be achieved in a cost effective and cost-beneficial manner.

With all of this in mind, in 2012, EU policy-makers and regulators funded a research project on the 'DEVelopment Of innovative Tools for understanding marine biodiversity and assessing good Environmental Status' (DEVOTES: www.devotes-project.eu), under the 7th Framework Programme 'The Ocean of Tomorrow' Theme. The call for projects stated that the expected impacts from accepted proposals should "contribute to the implementation of the Marine Strategy Framework Directive (MSFD) and associated Commission Decision on Good Environmental Status (GES) and strengthen the knowledge base necessary to address sustainable management of seas and oceans resources". This means that any selected project should contribute to bridge the gap between policy (i.e. MSFD) and science (in this case, creation of indicators, models, assessment tools, etc.), by increasing the knowledge necessary to assess the marine environmental status in an effective manner.

During the preparation of the proposal, we were well aware of the need of building a multidisciplinary team with a focus on strong collaboration among European institutions, regional seas as well as overseas partners, to achieve much needed synergies in research. Hence, more than 200 scientists from 23 institutes and 15 countries, including observers from the United States and an Advisory Board with members from Canada, the European Commission and European Regional Seas Conventions, have contributed to DEVOTES. Further collaboration with other European and national projects was initiated during the four year lifespan of DEVOTES (2012-2016) (see Mea et al. (2016), in this eBook). This internal and external collaboration has resulted in many scientific sessions organized in international conferences, several summer schools, hundreds of contributions to conferences and to date over 150 scientific papers (see details in Mea et al., 2016).

From the beginning of the project we were committed to publishing our research in open access outlets, making our results available to scientists, stakeholders, policy-makers and the society at large. As such, all DEVOTES deliverables are public (http://www.devotes-project.eu/deliverables-and-milestones/), the software and tools produced under DEVOTES are freely available (www.devotes-project.eu/software-and-tools), and all our papers are in gold and green open access (http://zenodo.org/collection/user-devotes-project). However, with the aim of bridging the communication gap between science and policy, the scientific knowledge generated in DEVOTES has also been communicated to policy makers through policy briefs, local press releases, fit-for-purpose workshops/webinars and conferences, etc. (see details in Mea et al., 2016).

Additionally, we took the view that a Research Topic in *Frontiers in Marine Science* would would be an ideal platform for synthesizing and promoting the results from DEVOTES as well as other projects developing tools to improve marine management, and putting these into a global context. We invited the scientific community to contribute their research worldwide to advance the knowledge on assessing health status of marine ecosystems. This Research Topic is the result of this effort, in which we have included investigations from the DEVOTES project published in *Frontiers in Marine Science* between 2014 and 2016 (Borja, 2014; Carstensen, 2014; Andersen et al., 2014; Galparsoro et al., 2014; Borja et al., 2014, 2016a), together with new syntheses and reviews (Smith et al., 2016; Mea et al., 2016; Borja et al., 2016b) and original research (Korpinen and Andersen, 2016; Patricio et al., 2016a, 2016b; Ferrera et al., 2016; Aylagas et al., 2016; Queirós et al., 2016; Uusitalo et al., 2016). We also included studies from external research groups which complement the DEVOTES studies (Chartrand et al., 2016; Callaway, 2016; Noble et al., 2016).

The first edition of this eBook will shortly be completed with additional manuscripts currently under review. It is structured as follows:

- An introduction, which explains the background of the Research Topic and introduces the grand challenges in marine ecosystems ecology (Borja, 2014), some of which have been addressed within DEVOTES and are included in this eBook.

- The legal and administrative framework of marine activities and management, including the efforts made in the past 20 years in developing a unified framework for marine management (Patricio et al., 2016a); the conceptual models used in managing the marine environment (Smith et al., 2016); and a global review of cumulative pressure and impact assessment (Korpinen and Andersen, 2016).

- The need for fit-for-purpose monitoring by first understanding and assessing current European Marine Biodiversity Monitoring Networks (Patrício et al., 2016b), then developing innovative monitoring methods such as: the use of new molecular methods in monitoring picoplankton (Ferrera et al., 2016) and macroinvertebrates (Aylagas et al., 2016); and the use of historical data in studying benthic fauna (Callaway, 2016). All of this with the aim of ensuring sustainable provision of marine ecosystem services (Carstensen, 2014).

- The need for good monitoring data linked to indicators to assess the environmental status of marine ecosystems. Hence, an objective framework to test the quality of candidate indicators of good environmental status is presented (Queirós et al., 2016). However, indicators need adequate thresholds, and described in a study on thresholds to prevent dredging impacts on seagrasses (Chartrand et al., 2016). In addition, the assessment of ballast water exchange compliance is discussed (Noble et al., 2016).

- A solid framework to assess environmental status in an integrative way is required. To this end, different ways in which multiple ecosystem components can be integrated in holistic evaluations (Borja et al., 2014) and a revision of currently available methods to undertake such integrated assessments (Borja et al., 2016a) are presented. In addition, the basis for a new assessment tool was set (Andersen et al., 2014) and this new tool (Nested Environmental status Assessment Tool: NEAT) was tested in 10 case studies across all European seas (Uusitalo et al., 2016).

- The socio-economic perspective of this work deserves attention as well as the ability of marine habitats to provide ecosystem services, which in turn provide societal benefits, as presented by Galparsoro et al. (2014).

- And, last but not least, we need to disseminate our important results after four years of intense research beyond the scientific community and improve the knowledge transfer between researchers and policy makers. Therefore, we studied ways to enhance the effectiveness of research results communication (Mea et al., 2016) and how DEVOTES has contributed to filling in the gaps between policy and science for assessing the health status of marine systems, including the main challenges for the future (Borja et al., 2016b).

We hope that all readers of this eBook will find the collection of papers useful in their daily work, through selecting appropriate indicators, implementing and improving monitoring networks, modelling marine systems, or assessing the status in an integrative way. Our aim with this eBook has been to convey the outcome of the DEVOTES collaborative and multidisciplinary work to a broad audience, including scientists, policy-makers, environmental managers, stakeholders and the public in general. Although bridging science and policy will always remain a challenge, our hope is that with this eBook the gap has been reduced. We are confident that you will enjoy reading these papers as much as we did writing them!

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Development of innovative tools for understanding marine biodiversity and assessing good environmental status

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Grand challenges in marine ecosystems ecology

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INTRODUCTION

The study of marine ecosystems has become a hot research topic in recent times. In fact, the number of manuscripts including the words "marine ecosystems" published since 1970 has immensely increased reaching between 1100 and 1500 articles per year in the past five years (**Figure 1**). Based on the keywords used in these manuscripts, the most frequent topics can be grouped into: (i) marine ecosystems (28.8% of the papers); (ii) biodiversity (26.6%), used as keyword at any level of organization, such as bacteria, phytoplankton, zooplankton, benthos, fishes, mammals, seabirds, etc.; (iii) functionality (10.7%), including aspects such as ecosystem function, biomass, food-webs, primary and secondary production, etc.; (iv) environmental research (9.7%), including pollution, environmental monitoring, human pressures, impacts, etc.; (v) structural parameters (6.6%) such as abundance, richness, diversity; (vi) climate change (3.4%); (vii) ecology (3.4%); (viii) systems management (3.2%); (ix) genetic and genomic issues (1.6%); (x) protection (1%); (xi) ecosystem modeling (0.9%); and (xii) others (4.5%).

Taking into account the large number of papers published in recent years, several grand challenges can be identified for future research within the field of marine ecosystem ecology and as outlined below.

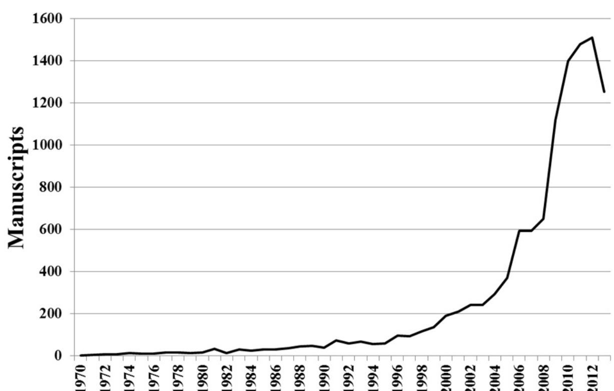

FIGURE 1 | Number of manuscripts published under the term: "marine ecosystem," appearing in the abstract, title or keywords, since 1970, within the Science Citation Index journal (consulted in SCOPUS, on 17th November 2013).

GRAND CHALLENGE 1: UNDERSTANDING THE ROLE OF BIODIVERSITY IN MAINTAINING ECOSYSTEMS FUNCTIONALITY

Currently, the global species extinction rate far exceeds that of speciation, this difference being the primary driver for change in global biodiversity (Hooper et al., 2012). The rate of biodiversity loss is one of the 10 planetary boundaries within which humanity can operate safely that has already been exceeded (Rockström et al., 2009). The effects of this global decline in biodiversity provide evidence of its importance in sustaining ecosystem functioning and services and preventing ecosystems from tipping into undesired states (Folke et al., 2004).

Historically, researchers have investigated ecosystems focusing on individual or few components of biodiversity, i.e., microbes, phytoplankton, zooplankton, macroalgae, macroinvertebrates, fishes, mammals, seabirds, etc., trying to understand individual species' roles. However, it is now recognized that understanding the entire ecosystem requires the study of all biodiversity components, from the genetic structure of populations, to species, habitats and ecosystem integrity, including food-webs and complex bio-physical interrelationships within the system.

Thompson et al. (2012) emphasize that food-web ecology will act as an underlying conceptual and analytical framework for studying biodiversity and ecosystem function, if the following challenges are addressed: (i) relating food-web structure to ecosystem function; (ii) combining food-web and ecosystem modeling; (iii) transitioning from individual traits to ecosystem function; (iv) incorporating space and time in studies; and (v) understanding the effects of biodiversity loss on ecosystem function.

The study of the ecological function of biodiversity is very recent; yet, it has been recognized to have fundamental implications for predicting the consequences of biodiversity loss (Benedetti-Cecchi, 2005). Species in an ecosystem can be functionally equivalent, meaning that they play the same role. As such, these functionally equivalent species can be grouped together as functional types (i.e., guilds, trophic groups, structural groups, ecological groups, traits). Other key attributes of biodiversity organization, such as the density mass–relationship between abundance and body size, have become a major research area. These attributes relate to food webs, determined by the trophic position, predator–prey relationships, and energy balance. Theoretically, a higher number of functional group types will provide higher functional biodiversity organization to the system, and thus, contribute to more stable and resilient ecosystems (Tomimatsu et al., 2013).

Despite the importance of this question, the relationship between diversity and stability is still being resolved. As with many biodiversity-related topics, there are different ways of expressing stability. One way is to define it as the ability of a system to return to its original state after being disturbed (i.e., resilience), so how quickly it can return and how large a disturbance it can return from are key variables (Elliott et al., 2007). Another definition is how resistant to change the system is in the first place. No matter which definition is used, there are definite trends that appear.

Finally, a major issue in maintaining the functionality of ecosystems comes from invasive species, which can dramatically disturb stable systems thereby impacting ecosystem services (Sorte et al., 2010; Vilà et al., 2010). Methods to detect and control this biological pollution are therefore needed (Olenin et al., 2011).

GRAND CHALLENGE 2: UNDERSTANDING RELATIONSHIPS BETWEEN HUMAN PRESSURES AND ECOSYSTEMS

Global biodiversity is threatened by human activities which are increasingly impacting marine ecosystems (Halpern et al., 2008). These impacts are usually cumulative and can lead to degrading habitats and ecosystem functionality (Ban et al., 2010). In some seas, such as the Mediterranean and Black Sea, less than 1% of the surface is considered unaffected by human disturbance with most of the surface affected by cumulative impacts (Micheli et al., 2013). There is evidence that the likelihood of regime shifts may increase as a result of reduced ecosystem resilience through a decrease in diversity, functional groups of species or trophic levels, thereby impacting ecosystems (with waste, pollutants and climate change) and altering the magnitude, frequency, and duration of disturbance regimes (Folke et al., 2004).

Current socio-ecological theories consider humans as part of the marine ecosystem (Livingston et al., 2011). Hence, understanding the relationships between human activities and their various impacts on marine ecosystems represents another grand challenge to be discussed within the specialty section of Marine Ecosystem Ecology.

GRAND CHALLENGE 3: UNDERSTANDING THE IMPACT OF GLOBAL CHANGE ON MARINE ECOSYSTEMS

Sea waters are getting warmer, sea-level rise is accelerating and the oceans are becoming increasingly acidic (Stocker et al., 2013). From a database of 1735 marine biological responses to global change, Poloczanska et al. (2013) determined that 81–83% of all observations for distribution, phenology, community composition, abundance, demography and calcification across taxa and ocean basins were consistent with the expected impacts of climate change on marine life (Richardson et al., 2012).

As there is an insufficient understanding of the capacity for marine organisms to adapt to rapid climate change, Munday et al. (2013) emphasize that an evolutionary perspective is crucial to understanding climate change impacts on our seas and to examine the approaches that may be useful for addressing this challenge.

We need also a deeper understanding of the climate change impact on body size and the cascading implications on ecosystem functioning, considering the recent attempt of applying metabolic theory on modeling the biosphere. Hence, organisms often have smaller body sizes under warmer climates, and body size is a major determinant of functionality of the ecosystems, as commented above. Therefore, by altering body sizes in whole communities, current warming can potentially disrupt ecosystem function and services (Edeline et al., 2013).

In addition, our understanding of the linkages between climate change and anthropogenic disturbances needs to be improved. Borja et al. (2013b), investigating the combined effects of human pressures (i.e., exploitation and waste discharges) and environmental variables (i.e., light, waves, temperature) in macroalgae over a long-term series, demonstrated that in impacted areas macroalgae are more vulnerable to environmental changes and that their resilience is reduced. In turn, there is clear evidence that marine reserves enhance resilience of ecosystems to climatic impacts (Micheli et al., 2012).

As determined by Philippart et al. (2011), a better understanding of potential climate change impacts can be obtained by: (i) modeling scenarios at both regional and local levels; (ii) developing improved methods to quantify the uncertainty of climate change projections; (iii) constructing usable climate change indicators; and (iv) improving the interface between science and policy formulation in terms of risk assessment. These factors are essential to formulate and inform better adaptive strategies to address the consequences of climate change.

GRAND CHALLENGE 4: ASSESSING MARINE ECOSYSTEMS HEALTH IN AN INTEGRATIVE WAY

Assessing the status of the oceans requires tools that allow us to define marine health across different marine habitats. Such tools have been developed in recent years, including ecological indicators to be applied to different ecosystem components (Birk et al., 2012; Halpern et al., 2012). One of the current challenges is to clearly understand what good status or good health is/means in marine systems and how we know when it has been attained (Borja et al., 2013a; Tett et al., 2013). This way, integrating knowledge across different ecosystem components and linking physical, chemical and biological aspects when assessing the status of marine systems is crucial for accurate evaluations (Borja et al., 2009, 2011).

However, one of the most critical issues when assessing the health status of marine ecosystems relates to the setting of adequate reference conditions and/or environmental targets to which monitoring data should be compared (Borja et al., 2012). These targets should be set taking the ecological characteristics of the studied ecosystems into account.

GRAND CHALLENGE 5: DELIVERING ECOSYSTEM SERVICES BY CONSERVING AND PROTECTING OUR SEAS

Marine ecosystems provide numerous goods and services (Barbier et al., 2012), such as biogeochemical services (e.g., carbon sequestration), nutrient cycling, coastal protection (e.g., provided by coral reefs or phanerogams), food provision (e.g., fisheries), and grounds for tourism, etc. (Costanza et al., 1997). Despite the important role of such goods and services and albeit quickly attracting more attention, their study and their associated monetary value (often demanded to support conservation efforts) is still limited, particularly for the high seas and deep water habitats (Beaumont et al., 2007; Barbier et al., 2011; Braat and de Groot, 2012; Van den Belt and Costanza, 2012; Liquete et al., 2013; Thurber et al., 2013). Furthermore, recent debates have raised the question whether all ecosystem services can or should be quantified in monetary terms, when the public finds such values difficult to relate to.

It has been suggested that ecosystem services of high value critically depend on biodiversity (EASAC, 2009). As biodiversity loss is accelerating, maintaining biodiversity and healthy ecosystem services should be a priority when investigating, conserving and managing marine systems.

In marine management, Marine Protected Areas (MPAs) are an important tool for conserving and protecting biodiversity, by enhancing ecosystem resilience and adaptive capacity (Roberts et al., 2003; García-Charton et al., 2008). They allow for the mitigation of anthropogenic factors, such as overfishing or habitat destruction within their boundaries, by means of management or prohibition (Roberts et al., 2001; Mumby et al., 2006). Not only MPAs, but also the protection of near-natural ecosystems are very good strategies for managing climate change-related stressors and preserving biodiversity (Heller and Zavaleta, 2009).

Additional important issues in marine protection include the reduction of habitat fragmentation (Didham, 2010; Didham et al., 2012), determining the vulnerability of threatened species and habitats (Le Quesne and Jennings, 2012), and the study of connectivity between habitats and species distribution, which is a critical factor in maintaining habitat quality (Berglund et al., 2012).

GRAND CHALLENGE 6: RECOVERING ECOSYSTEM STRUCTURE AND FUNCTIONING THROUGH RESTORATION

Most estuarine, coastal and offshore waters worldwide have experienced significant degradation throughout the past three centuries (Lotze, 2010) and investments in marine protection have not been totally effective. Hence, ecological restoration is becoming an increasingly important tool to manage, conserve, and repair damaged ecosystems, as stated by Hobbs (2007).

Measuring effectiveness of restoration at habitat, community, or ecosystem level is not easy, and requires a focus on restoration of processes and functionality, rather than studying the recovery of particular species (Verdonschot et al., 2013). Thus, according to Borja et al. (2013c), restoration efforts should rely on what is known from theoretical and empirical ecological research on how communities and ecosystems recover in structure and function through time. Hence, studies on dispersal, colonization dynamics, patch dynamics, successional stages, metapopulations theory, etc., are needed for a deeper knowledge of recovery processes (Borja et al., 2010). This research will provide evidences to enhance restoration success of complex systems (Verdonschot et al., 2013).

GRAND CHALLENGE 7: MANAGING THE SEAS USING THE ECOSYSTEM APPROACH AND SPATIAL PLANNING

The management of marine systems, including the assessment of their overall health status, is increasingly carried out by applying ecosystem-based approaches (Borja et al., 2008). After all, the protection and conservation of marine ecosystems, together with the sustainable use of the services they provide, are of fundamental importance to the maintenance of global marine health (Tett et al., 2013). The goal of ecosystem-based management is to maintain an ecosystem in healthy, productive, and resilient conditions so that it can provide the services needed for the well-being of society (Yáñez-Arancibia et al., 2013). The guiding principles for ecosystem-based management are founded on the idea that ocean and coastal resources should be managed to reflect the relationships among all ecosystem components, including humans, as well as the resulting socioeconomic impacts (Yáñez-Arancibia et al., 2013).

In addition to the need for better management tools, the increasing anthropogenic impacts on marine waters (e.g., fisheries, aquaculture, shipping, renewable energies, recreation, mining, etc.) has promoted the discussion on how to manage and to conserve marine resources sustainably (Collie et al., 2013). Marine Spatial Planning, as defined by Ehler and Douvere (2009), is a management tool that attempts to balance conservation efforts with increasing demands on marine resources, which, together with the ecosystem-based approach, relies on a multidisciplinary approach integrating sociological, economic and ecological components (Qiu and Jones, 2013; Stelzenmüller et al., 2013).

GRAND CHALLENGE 8: MODELING ECOSYSTEMS FOR BETTER MANAGEMENT

The specificities of oceans when compared with terrestrial systems (see Norse and Crowder, 2005), and the increasingly complex approaches to investigate ecosystems at an integrative level requires the use of computer models (e.g., hydrodynamic, habitat suitability models, ecosystem models, etc.) for a better understanding of the processes, functioning and interrelationships among ecosystem components (Fulton et al., 2004). As a result, the use of species, ecological niche, habitat and ecosystem models has dramatically increased in recent years (Elith and Graham, 2009; Ready et al., 2010).

To guide conservation actions more effectively, the use of species distribution models has been recommended (Guisan et al., 2013), for example for studies on biological invasions, the identification of critical habitats, etc.

CONCLUSION

To adequately address the abovementioned grand challenges in Marine Ecosystem Ecology, effective long-term monitoring of populations and communities is required to understand marine ecosystem functioning and its responses to environmental and anthropogenic pressures (Stein and Cadien, 2009). However, monitoring programs often neglect important sources of error (e.g., the inability of investigators to detect all individuals or all species in a surveyed area) and thus can lead to biased estimates, spurious conclusions and false management actions (Katsanevakis et al., 2012). One of the newest ways to get reliable, verifiable, efficient and cost-effective monitoring of biodiversity is metabarcoding (Bourlat et al., 2013; Ji et al., 2013).

In addition to the acquisition of information on a regular basis, complete maps of habitats, ecosystem services, etc., are needed for a better understanding of spatial ecology and marine management (Brown et al., 2011). All this information requires data integration of the different ecosystem components in order to understand large-scale patterns and long-term changes (Stocks et al., 2009; Vandepitte et al., 2010).

Finally, the movement toward open access to scientific data and publications provides greater access to datasets and current research, which has the potential to result in better spatial and temporal analyses, by using existing information in a much more effective way through Information and Communication Technologies (i.e., e-Science). Make data open, accessible online in a standard format available for aggregation, integration, analysis and modeling, is a crucial step to boost the development of marine ecosystem ecology, to address the above highlighted challenges, and to move toward the frontiers of marine science (see Baird et al., 2011). Therefore, Frontiers in Marine Ecosystem Ecology promotes open access to data and information to enhance collaborations, whilst discussing hot marine topics and addressing the grand challenges described here.

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Legal framework of marine activities and management

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REVIEW
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DPSIR—Two Decades of Trying to Develop a Unifying Framework for Marine Environmental Management?

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Determining and assessing the links between human pressures and state-changes in marine and coastal ecosystems remains a challenge. Although there are several conceptual frameworks for describing these links, the Drivers-Pressures-State change-Impact-Response (DPSIR) framework has been widely adopted. Two possible reasons for this are: either the framework fulfills a major role, resulting from convergent evolution, or the framework is used often merely because it is used often, albeit uncritically. This comprehensive review, with lessons learned after two decades of use, shows that the approach is needed and there has been a convergent evolution in approach for coastal and marine ecosystem management. There are now 25 derivative schemes and a widespread and increasing usage of the DPSIR-type conceptual framework as a means of structuring and analyzing information in management and decision-making across ecosystems. However, there is less use of DPSIR in fully marine ecosystems and even this was mainly restricted to European literature. Around half of the studies are explicitly conceptual, not illustrating a solid case study. Despite its popularity since the early 1990s among the scientific community and the recommendation of several international institutions (e.g., OECD, EU, EPA, EEA) for its application, the framework has notable weaknesses to be addressed. These primarily relate to the long standing variation in interpretation (mainly between natural and social scientists) of the different components (particularly P, S, and I) and to over-simplification of environmental problems such that cause-effect relationships cannot be adequately understood by treating the different DPSIR components as being mutually exclusive. More complex, nested, conceptual models and models with improved clarity are required to assess pressure-state change links in marine and coastal ecosystems. Our analysis shows that, because of its complexity, marine assessment and management constitutes a “wicked problem” and that there is an increasing need for a unifying approach, especially with the implementation of holistic regulations (e.g., European framework Directives). We emphasize the value of merging natural and social sciences and in showing similarities across human and natural environmental health. We show that previous approaches have adequately given conceptual and generic models but specificity and quantification is required.

Keywords: biodiversity, conceptual framework, drivers, pressures, state, impacts, response, environmental assessment

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INTRODUCTION

The highly-complex marine system has a large number of interrelated processes acting between its physical, chemical, and biological components. Many diverse human activities exert pressure on this complex environment and the cumulative environmental effects of these activities on the system varies according to the intensity, number and spatial and temporal scales of the associated pressures. There is an increasing need to demonstrate, quantify predict and communicate the effects of human activities on these interrelated components in space and time (Elliott, 2002). The study and management of marine systems therefore requires information on the links between these human activities and effects on structure, functioning and biodiversity, across different regional seas in a changing world. It also requires the need to merge approaches from natural and social sciences in structuring and solving the problems created by human activities in the seas (Gregory et al., 2013).

Conceptual models are needed to collate, visualize, understand and explain the issues and problems relating to actual or predicted situations and how they might be solved. These models can be regarded as organizational diagrams, which bring together and summarize information in a standard, logical and hierarchical way. Since the early 1990s, Pressure-State-Response (PSR) frameworks have been central to conceptualizing marine ecosystem risk analysis and risk management issues and then translating those for stakeholders, environmental managers and researchers. In this context, the pressures cause the changes to the system, the state changes are the unwanted changes and the responses are what society does to remove, minimize, or accommodate the changes. Hence, it is axiomatic that society has to be concerned about the risks to the natural and human system posed by those pressures (thus needing risk assessment) and then it is required to act to minimize or compensate those risks (as risk management) (Elliott et al., 2014).

It is apparent that one of the key current conceptual frameworks in widespread use, the Drivers-Pressures-State change-Impact-Response (DPSIR) framework (see **Figure 1A**—original concept and definitions from EC, 1999), has developed since the 1990s as the basis for most conceptual approaches addressing pressure-state change links. It is policy-oriented and provides a framework for categorizing a problem domain, along the cause-effect chain. The DPSIR framework was developed from the PSR framework initially proposed by Rapport and Friend (1979), and adapted and largely promoted by the OECD (Organization for Economic Cooperation and Development) for its environmental reporting (OECD, 1993). Several international organizations, such as US Environmental Protection Agency (EPA, 1994), UNEP (1994) and the EU have also adopted the framework, the latter noting that this was the most appropriate way to structure environmental information (EC, 1999). Within the EU, Eurostat focuses on Response (the societal mechanisms effecting ecosystem management, in particular, expenditure on environmental protection), Driving forces (environmentally relevant sectoral trends, for example, societal need for and food) and Pressure (e.g., resource exploitation trends). Indicators of State and Impact are the domain of the European Environment Agency (EC, 1999) which is required to communicate the state of the environment for policy-makers. DPSIR has thus been used with increasing frequency for problem solving both by natural and social scientists and they have further refined/defined and applied DPSIR and its derivatives in an on-going process tailored to many different applications.

Gari et al. (2015) recently reviewed 79 published and gray literature sources involving eight DPSIR derivatives for coastal social-ecological systems. More recently, Lewison et al. (2016) reviewed many papers covering 24 relevant DPSIR coastal zone articles. Both publications point out limitations and in particular differences in the terminology or definitions used by different authors. Important differences in definitions particularly concerning States and Impacts, had led to the "modified DPSIR" (mDPSIR) of the ELME EU FP6 project. Within mDPSIR the Impact category was restricted to impacts on human systems thus leading in turn to the definition of the DPSWR framework in the KNOWSEAS FP7 project, where Cooper (2013) replaced Impact with Welfare. However, it has been suggested that it is the "impact on human welfare" rather than "welfare" *per se* that is important hence leading to the most recent DAPSI(W)R(M) derivative (Wolanski and Elliott, 2015; Scharin et al., 2016) (**Figure 1B**). In another modification, used by social scientists, DPSIR has been related to Goods and Services through EBM-DPSER where Ecosystem Based Management (EBM) is directly related to Driver-Pressure-State-Ecosystem Service-Response (Kelble et al., 2013) or the Ecosystem Services and Societal Benefits (ES&SB) linked-DPSIR approach (Atkins et al., 2011). A further development of DPSIR in the area of human health has been the DPSEEA framework comprising Driving forces-Pressures-State-Exposure-Effect-Action (and sometimes DPSEEAC, where "C" relates to Context), a framework used primarily in risk assessments for contaminants and developed by the World Health Organization (von Schirnding, 2002). Given that such a framework requires indicators to determine whether management actions are effective, successful and sustainable (Elliott, 2011), a further development was in creating indicators such as those of child environmental health using the MEME framework (many-exposures many-effects); this therefore progressed from the linear and pollution-based view of DPSEEA (and other) frameworks (Briggs, 2003).

Given the above history and confusion, as part of the EU funded DEVOTES project (see http://www.devotes-project.eu), we have comprehensively reviewed marine/coastal environmental investigations concerned with the DPSIR framework and its derivatives. We have furthermore assessed its applications, habitats addressed, geographical use, problems and developments, and the general advantages and disadvantages of using the framework to address marine issues. Our aim was to establish the extent to which DPSIR as an overarching framework has been applied to marine and coastal ecosystems and to identify factors which either facilitate or hinder its application. In this way, we focus on the ability and adequacy of the DPSIR framework to analyze and explain the relationships between human uses of the seas and the resulting problems, their management and the communication of these to interested

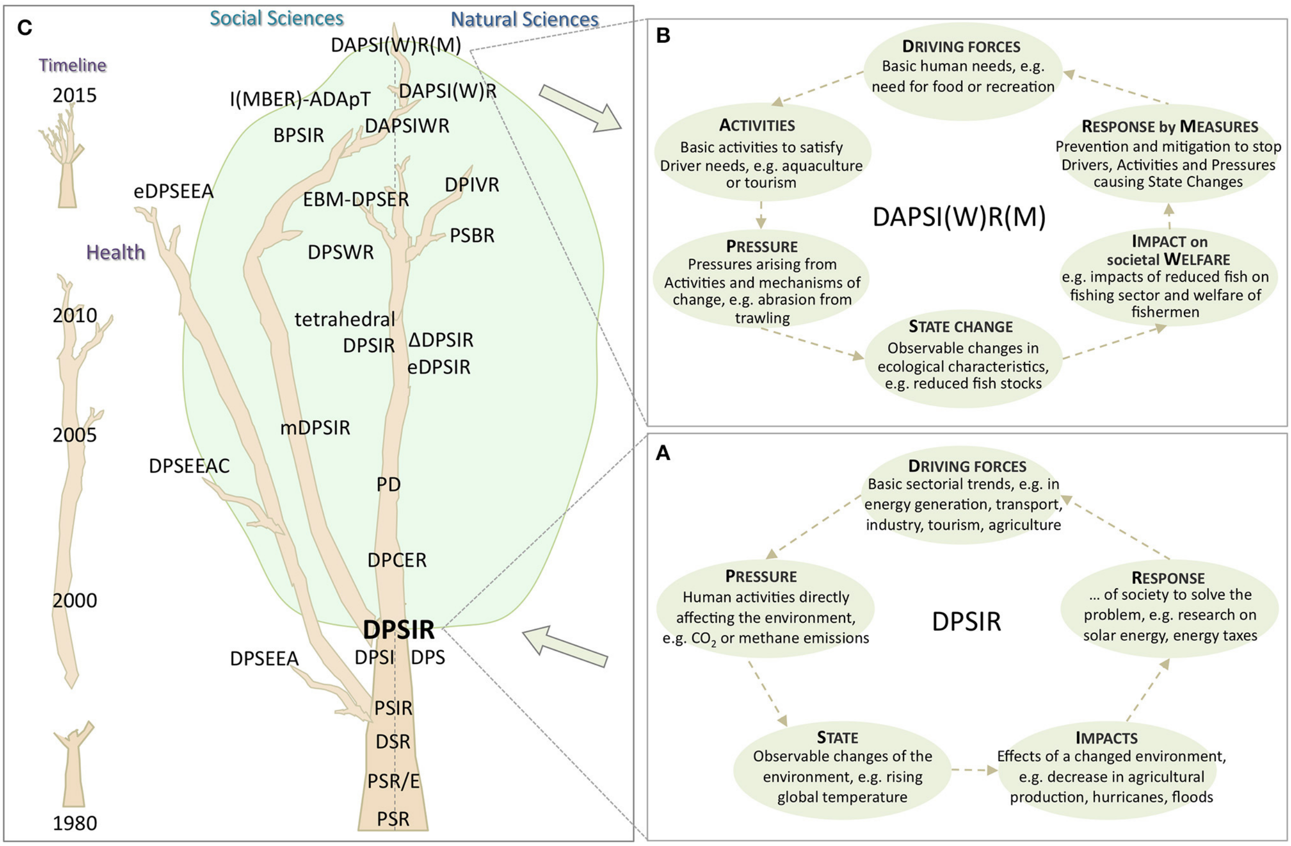

FIGURE 1 | DPSIR and derivatives development. (A) DPSIR first elaboration, redrawn from the original EU framework (EC, 1999), **(B)** DAPSI(W)R(M), top of the tree evolution of DPSIR (as defined in Scharin et al., 2016), **(C)** timeline and development/relationship of DPSIR and derivatives.

stakeholders. To our knowledge, this is the first study that summarizes the use of DPSIR in marine ecosystems.

MATERIALS AND METHODS

This comprehensive review of the available literature concerned with the DPSIR framework, its "derivatives" and other related frameworks. We used the following search keywords both singly and in combination: DPSIR, PSR, Drivers + Pressures + State + Impacts + Responses, State change, conceptual framework, Marine and Coastal. We conducted primary searches using Web of Science, ScienceDirect, Scopus and Google Scholar and then checked the reference lists of the previous review papers. We only considered publications published in English. We furthermore compiled projects starting with an initial list of European projects where DPSIR was known to be used as a conceptual framework and then we expanded the search using the same keywords used for publications plus the word "project." Our keyword-based screening was narrowed according to the text in the abstracts. We retained documents when the abstract explicitly mentioned the DPSIR framework or any derivative and was linked with coastal or marine ecosystems. Although this review focused primarily on research projects and publications dealing with these ecosystems, the scope broadened to include both projects and publications that present or discuss the framework, regardless of its application to specific case studies and studies that address biodiversity (*sensu lato*) under the scope of DPSIR.

The 152 studies retained for the review included research papers, review papers, essays, short communications, viewpoint papers, seminar papers, discussion papers, journal editorials, policy briefs, conference long abstracts, monographs, technical reports, manuals, synthesis or final project reports and book chapters (**Figure 2A**). The studies were collated and, after detailed reading, each reference was categorized by "Study site," "Habitat," "Region," "Framework/Model type," "Issue/problem addressed by the study," "Implementation level" and "Type of publication." Appendix 1 in Supplementary Material presents the final list of references and their classification according to the previous categories.

The analysis also considered research projects from 1999 onwards and showed that at least 27-research projects focusing on coastal and marine habitats have used (or are using) the DPSIR framework and/or derivatives as part of their conceptual development phases. Appendix 2 in Supplementary Material shows the final list of projects that were considered, categorized by "Acronym," "Title," "Duration," "Funding institution," "Region," "General objective" of the project, "Framework" used, "Keywords," "Website" and some examples of "Output

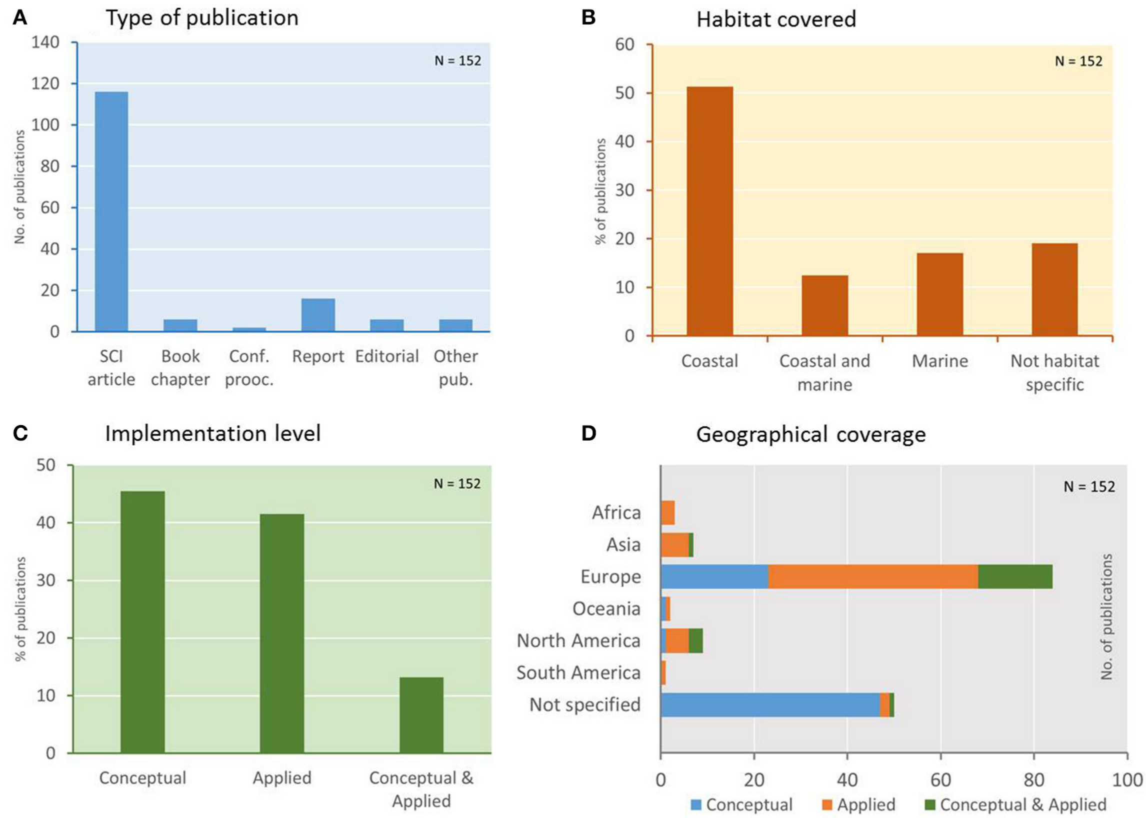

FIGURE 2 | (A) Types of publication; **(B)** Habitats covered; **(C)** Implementation level; and **(D)** geographical coverage.

references." A further column gives complementary details for the projects where available.

Box 1 shows the 25 frameworks found in the review and the general components of each conceptual model.

RESULTS

Published Investigations

Despite the increasing popularity of the DPSIR framework and derivative models among the scientific community since the early 1990s, and the recommendations of OECD (1993), EPA (1994), EEA (1999), and EC (1999) for its application, few studies have focused on the marine habitat (**Figure 2B**). From our comprehensive review, only 26 studies exclusively cover this habitat and from these, only eight illustrate concrete case studies [German Exclusive Economic Zone (Fock et al., 2011); German waters of the North Sea (Gimpel et al., 2013); Baltic Sea, Black Sea, Mediterranean Sea, and North East Atlantic Ocean (Langmead et al., 2007, 2009); Baltic Sea (Andrulewicz, 2005); North and Baltic Sea (Sundblad et al., 2014); Northwestern part of the North Sea (Tett et al., 2013) and Florida Keys and Dry Tortugas (Kelble et al., 2013)]. The remaining 18 studies are either explicitly conceptual or illustrate the framework with generic situations/issues. For example, Elliott (2002) examined offshore wind power and Ojeda-Martínez et al. (2009) studied the management of marine protected areas.

In addition to studies exclusively focusing on marine habitats, 19 others focused simultaneously on marine and coastal habitats (13 of them applied). These cover the Mediterranean region (Casazza et al., 2002), Portuguese marine and coastal waters (Henriques et al., 2008), German North Sea (Lange et al., 2010), West coast of Schleswig-Holstein (Licht-Eggert, 2007), Baltic Sea (Lundberg, 2005; Ness et al., 2010; Lowe et al., 2014), Dutch Wadden Sea region (Vugteveen et al., 2014), UK waters (Rogers and Greenaway, 2005; Atkins et al., 2011), the North East Atlantic (Turner et al., 2010) and the Black Sea (Hills et al., 2013).

Approximately half of the references focus explicitly on coastal habitats (e.g., estuaries, coastal lagoons, entire basins) and half of these are solid case studies where, to a lesser or greater extent, the DPSIR framework or derivatives were applied (for examples, see **Box 2**). The remaining references ($N = 29$) are not habitat-specific (**Figure 2B**). Approximately 45% of the studies are conceptual (i.e., defining or reviewing the frameworks, using DPSIR and derivatives as reporting outline or as a framework for selecting environmental indicators, assessing biodiversity loss, etc.) (**Figure 2C**).

It is also of note that most publications refer to the use of DPSIR as a framework for specific issues (**Box 2**), for gaining greater understanding, as a research tool, for capturing and

BOX 1 | FRAMEWORKS FOUND IN THE REVIEW AND THEIR BASIC COMPONENTS.

- BPSIR: Behavior - Pressure - State - Impact – Response
- DAPSI(W)R: Drivers – Activities – Pressures – State (change) – Impacts on human Welfare – Response
- DAPSIWR: Drivers – Activities – Pressures – State (change) – Impacts on environment – Impacts on welfare – Response
- DAPSI(W)R(M): Drivers – Activities – Pressures – State change – Impacts (on human Welfare) Response (using Measures)
- DPCER: Driver - Pressure - Chemical state - Ecological state – Response
- DPS: Driver - Pressure – State
- DPSEA: Driver - Pressure - State - Effect – Action
- DPSEEA: Driver - Pressure - State - Exposure - Effect – Action
- DPSEEAC: Driver – Pressure – State – Exposure – Effect – Action – Context
- DPSI: Driver - Pressure - State – Impact
- DPSIR: Driver - Pressure - State - Impact – Response
- DPIVR: Drivers – Pressures – Impacts – Vulnerability - Response
- ΔDPSIR - Differential Drivers - Pressure - State - Impact – Response
- DPSWR: Driver - Pressure - State (change) - Welfare – Response
- DSR: Drivers - State – Response
- EBM-DPSER (or DPSER-EBM): Ecosystem Based Management/Driver - Pressure - State - Ecosystem service – Response
- eDPSEEA: ecosystems-enriched Driver - Pressure - State - Exposure - Effect – Actions
- eDPSIR: enhanced Driver - Pressure - State - Impact – Response
- I(MBER)-ADApT: Assessment based on Description, Response and Appraisal for a Typology
- mDPSIR: Driver - Pressure - State - Impact – Response
- PD: Pressures – Drivers
- PSBR: Pressure - State - Benefits – Response
- PSIR: Pressure - State - Impact – Response
- PSR/E: Pressure - State - Response – Effects
- Tetrahedral DPSIR: Driver - Pressure - State - Impact – Response (adapted)

BOX 2 | KEY AND RECENT PUBLICATIONS IN WHICH DPSIR AND DERIVATIVES HAVE BEEN USED.

Uses of DPSIR framework	Indicative references
Development and selection of indicators	Bowen and Ryley, 2003; EPA, 2008; Espinoza-Tenorio et al., 2010; Bell, 2012; Perry and Masson, 2013; Pettersson, 2015
Assessment of eutrophication	Bricker et al., 2003; Cave et al., 2003; Newton et al., 2003; Karageorgis et al., 2005; Lundberg, 2005; Nunneri and Hofmann, 2005; Pirrone et al., 2005; Rovira and Pardo, 2006; Trombino et al., 2007; Zaldívar et al., 2008; Gari, 2010; Garmendia et al., 2012
Assessment of the impact and vulnerabilities of climate change	Holman et al., 2005; Hills et al., 2013; Hossain et al., 2015
Fisheries and/or aquaculture management	Rudd, 2004; Mangi et al., 2007; Marinov et al., 2007; Viaroli et al., 2007; Henriques et al., 2008; Hoff et al., 2008 in Turner et al., 2010; Knudsen et al., 2010; Ou and Liu, 2010; Nobre et al., 2011; Cranford et al., 2012; Martins et al., 2012
Integrated coastal management	Turner et al., 1998b, 2010; EEA, 1999; Licht-Eggert, 2007; Mateus and Campuzano, 2008; Schernewski, 2008; Vacchi et al., 2014; Vugteveen et al., 2014; Dolbeth et al., 2016
Management of marine aggregates	Atkins et al., 2011; Cooper, 2013
Assessment of seagrass decline	Azevedo et al., 2013
Management of water resources	Giupponi, 2002, 2007; Mysiak et al., 2005; Yee et al., 2015
Assessment of wind farm consequences	Elliott, 2002; Lange et al., 2010
Ecosystem health assessment	Tett et al., 2013; Wang et al., 2013
Framing monitoring activities	Pastres and Solidoro, 2012
Synthesis of information related with ecosystem goods and services	Butler et al., 2014

communicating complex relationships, as a tool for stakeholder engagement, as the subject of reviews and as the subject for further tool/methodology development linked to policy making and decision support systems. For example, Cormier et al. (2013), using Canadian and European approaches, emphasized DPSIR as a Risk Assessment and Risk Management framework and recommend that ICES (International Council for the Exploration of the Sea) uses this as their underlying rationale for assessing single and multiple pressures.

This review shows clearly that the DPSIR framework and its extensions have mainly been used in a European context (**Figure 2D**). If we consider only those studies that specify a geographical location ($N = 100$), only 20% of the studies were performed in other regions (e.g., EPA, 1994, 2008; Bricker et al., 2003; Espinoza-Tenorio et al., 2010; Kelble et al., 2013; Perry and Masson, 2013; Cook et al., 2014; Fletcher et al., 2014; Yee et al., 2015 in North America; Bidone and Lacerda, 2004 in South America; Turner et al., 1998a; Lin et al., 2007; Ou and Liu, 2010; Nobre et al., 2011; Wang et al., 2013; Zhang and Zue, 2013; Hossain et al., 2015 in Asia; Walmsley, 2002; Mangi et al., 2007; Scheren et al., 2004 in Africa; Cox et al., 2004; Butler et al., 2014 in Oceania).

Research Projects

Since 1999, at least 27-research projects focusing on coastal and marine habitats have used (or are using) the DPSIR framework and/or derivatives as part of their conceptual development phases (Appendix 2 in Supplementary Material). Three of these projects had a scope beyond coastal and marine ecosystems, aiming to tackle large-scale environmental risks to biodiversity (e.g., ALARM), to contribute to the progress of Sustainability Science (e.g., THRESHOLDS) and to identify and assess integrated EU climate change policy (e.g., RESPONSES). They have been included in this review as their findings can extend to coastal and marine habitats. One of these projects (ResponSEAble, see Appendix 2 in Supplementary Material for more details) specifically addresses the human-ocean relationship and the need to encourage Europeans to treat oceans with greater respect and understanding (see **Box 3**).

Hence the DPSIR is a framework that several European projects have applied and/or developed but is less commonly the case in non-EU areas. From the many projects that used the framework or derivatives, only one was non-European funded. The USA National Oceanic and Atmospheric Administration Centre for Sponsored Coastal Ocean Research supported the MARES project that developed the EBM-DPSER framework (see Kelble et al., 2013; Nuttle and Fletcher, 2013).

In addition to the scientific context, the role played by the DPSIR framework and/or derivatives also varied markedly from project to project: ELME, KNOWSEAS, ODEMM, DEVOTES, and VECTORS have used the DPSIR framework extensively and some of these projects have developed and further modified the framework (e.g., ELME–mDPSIR and KNOWSEAS–DPSWR). However, this review encountered some difficulties mainly in relation to accessing information (see * in **Box 3**). In other projects, it has been difficult to find specific content even with a careful and thorough examination of websites, lists of deliverables and publications. The lack of easy open-access acts as a constraint to apply and explore further the knowledge gained by the application of the conceptual frameworks.

DISCUSSION

The DPSIR framework, as used widely in the literature, aims to act as a tool linking applied science and management of human uses (and abuses) of the seas. Because of this, and as shown here, it is necessary to define the framework and its terms and to show how the framework has been used, to indicate its advantages and benefits, as well as its disadvantages and anomalies. Most importantly there is the need to show whether it fulfills a role and whether it needs modifying and, if so, how it should be modified for future applications in an increasingly complex system of marine uses, users, threats, problems, and management repercussions. In particular, if successful, the DPSIR framework presents a simplified visualization and means of interrogating and managing complex cause-effect relationships between human activities, the environment, and society. It can therefore be used to communicate between disciplines (Tscherning et al., 2012), addressing the different aspects of environmental management (research, monitoring, mitigation, policy, and society) and between scientists, policy makers, and the public (Niemeijer and de Groot, 2008; Tscherning et al., 2012).

DPSIR—Advantages and Benefits as a Holistic Framework

DPSIR—A Wide-Ranging Tool Applicable to All Types of Environmental Problems

Through identifying the progressive chain of events leading to state change, impact, and response, the DPSIR framework and derivatives can potentially be applied to all types of environmental problems. For example, Fock et al. (2011) used PSR to link marine fisheries to environmental objectives concerning seafloor integrity in the German EEZ (Economic Exclusive Zone). Langmead et al. (2007) used mDPSIR to organize information relating habitat change, eutrophication, chemical pollution, and fishing in several European seas. Hills et al. (2013) used DPIVR to assess the impact of, and the vulnerability of marine and coastal ecosystems to, climate change. Lange et al. (2010) used DPSIR to analyse coastal and marine ecosystem changes related with offshore wind farming. Additionally, the framework and its derivatives, have been often used to select and develop indicators for environmental analysis (e.g., Casazza et al., 2002; Andrulewicz, 2005; Rogers and Greenaway, 2005) and inform management decisions (Kelble et al., 2013).

DPSIR—A Tool for Risk Assessment and Risk Management

While the DPSIR framework has been used for certain types of problems in the marine environment, the most important aspect is in tackling a set of hazards which, if they adversely affect human assets, economy and safety, become risks to society (Elliott et al., 2014). The hazards may be from natural sources, such as erosion patterns, tsunamis, or isostatic rebound due to geological phenomena. More importantly, from a societal view, they may be anthropogenic such as the over-extraction of material from the sea, the input of chemicals or the building of structures such as windfarms. Human actions may exacerbate the hazards and

BOX 3 | EU PROJECTS IN WHICH DPSIR AND DERIVATIVES HAVE BEEN USED IMPLICITLY OR EXPLICITLY.

Areas in which the framework is used	Indicative EU Project (∗ website no longer active)
To improve Integrated Coastal Zone Management and planning maritime safety	e.g., BLAST
integration of climate change into development planning	e.g., CLIMBIZ, RESPONSES, LAGOONS
To provide a roadmap to sustainable integration of aquaculture and fisheries	e.g., COEXIST
application of an ecosystem based marine management, the Ecosystem Approach to management or to fisheries	e.g., ODEMM, KNOWSEAS, CREAM
To integrate the marine and human system and assess human activity and its social, economic and cultural aspects	e.g., ELME∗, KNOWSEAS, VECTORS, ODEMM, BS-HOTSPOTS, PERSEUS, DEVOTES
To support scientifically the implementation of several European directives and legislation	e.g., ODEMM, LAGOONS, MULINO, SPICOSA, KNOWSEAS, PERSEUS, DEVOTES
To improve the knowledge of how environmental and man-made factors are impacting the marine ecosystems and are affecting the range of ecosystem goods and services provided	e.g., VECTORS, ODEMM, DEVOTES, SESAME∗, LAGOONS
To produce integrated management tools	e.g., MESMA, ODEMM, DITTY∗, MULINO, LAGOONS, DEVOTES
To look at spatial management and conflicts/synergies/trade offs	e.g., MESMA, COEXIST, ODEMM
To look at sectoral growth scenarios, sustainability, blue growth and the challenge of good environmental status	e.g., MEDTRENDS
To produce threat, risk and pressure assessment	e.g., ODEMM, DEVOTES
To produce new biodiversity indicators and Environmental Status assessment tools	e.g., DEVOTES
To produce engaging and informative story lines and tools about the oceans to raise interest and awareness among Europeans	e.g., ResponSEAble

lead to greater risks such as the removal of a protective saltmarsh or seagrass bed which otherwise could absorb energy and reduce erosion and the consequences of sea-level rise (Elliott et al., 2016). As such those human-induced hazards and risks emanate from activities and thus lead to the pressures as mechanisms resulting in adverse effects unless mitigated; consequently management responses as measures are required to address, mitigate or reduce those hazards and risks.

Each of those risks requires assessment, both cumulatively and in-combination thus requiring a rigorous framework that can accommodate multiple risks. Cumulative threats and pressures emanate from within one activity whereas in-combination threats and pressures arise from multiple activities occurring concurrently in an area. Therefore, once the risks are identified, by determining the source or cause of the threat and its consequences for the marine system, there needs to be a rigorous risk management framework (Cormier et al., 2013) which has to encompass a suite of measures by covering social, governance, economic, and technological aspects (Barnard and Elliott, 2015). This risk assessment and risk management framework thus especially encompasses the DPSIR approach in which the source and causes of risk are the Drivers and Pressures, the consequences are the State Change and Impacts and the risk is managed through the Responses (see Cormier et al., 2013).

DPSIR—A Stakeholder-Inclusive and Communication Tool for Implementing the Ecosystem Approach

DPSIR use has been adopted by and demonstrated to various actors, including research, academia, central and regional policy and decision makers, environmental NGOs, and the wider public. As an example, the EBM-DPSER model for the Florida Keys and Dry Tortugas is the agreed outcome of the joint efforts of over 60 scientists, agency resource managers, and environmental non-governmental organizations (Kelble et al., 2013). Various central administration bodies in Europe have used or are using the framework including, for example, the EEA, UNEP, and the Black Sea Commission (e.g., CLIMBITZ and BS-HOTSPOTS projects). UNEP used the framework as the base for organizing its State of the Environment assessment report (UNEP/MAP., 2012) by including an overview of major drivers in the Mediterranean, an analysis of the pressures, state and known impacts associated with each of the issues addressed by the Ecosystem Approach Ecological Objectives as well as major policy responses. Environmental NGOs have used the framework to present the main issues and to focus their need-for-change message to both the public and policy makers (e.g., WWF, MEDTRENDS project). Despite this, the level of detail depicted in these mostly conceptual applications of the DPSIR framework varies greatly. Most of the publications and projects included in this review do not go beyond the conceptual level although some of the conceptual models do include more details and/or more levels (e.g., Atkins et al., 2011). While O'Higgins et al. (2014) and Scharin et al. (2016) use the framework as a tool to analyse the relationship between human activities and their Impacts or to capture the information needed for marine management, Pettersson (2015) presents a case around eDPSIR and the Port of Gothenburg that includes development of indicators for factors influencing biodiversity and for the assessment of biodiversity itself. Pastres and Solidoro (2012), for the Venice lagoon, emphasize the importance of adopting a DPSIR approach to monitoring strongly supported by modeling tools and mathematical models as these can provide quantitative links between Pressures and State/Impacts. Furthermore, Cook et al. (2014) use detailed conceptual models (EBM-DPSER) together with expert opinion and matrix analyses to explore the direct and indirect relative impact of 12 ecosystem pressures on 11 ecosystem states and 11 ecosystem services.

DPSIR—Disadvantages and Anomalies

DPSIR—Restricted Coverage and Application

It is emphasized here that there is a widespread and increasing usage of DPSIR-type conceptual framework models in management and issue-resolving. Although many papers are conceptual, there are more case studies over time either used to describe an issue, thereby communicating a problem with an emphasis on the P-S link, where the natural scientists can apply a high degree of detail, or give the framework entirety across the whole cycle, solving problems through management with more involvement of social scientists, but less detail on the P-S links. In a more restricted study, Lewison et al. (2016) noted that only eight of the 24 DPSIR articles that they reviewed actively engaged decision-makers or citizens in their research, thereby completing a full cycle or involving all stakeholders. Bell (2012) emphasized that the challenge for DPSIR is to be both a precise Problem Structuring Method and of wide use to stakeholders.

It is of note that the analysis here clearly shows that the use of DPSIR is primarily European-based, also noted in the Lewison et al. (2016) review, with surprisingly sparse use elsewhere such as in the USA. This should not necessarily be regarded as a less-holistic or integrated approach to environmental issues, although it may be the result of the European framework directives guiding sustainability becoming increasingly complex, inclusive and integrated with respect to ecosystems, humans, and their activities (Boyes and Elliott, 2014). However, driving the European use is not just the institutional organizations of the EU, but also growth through parallel and sequential funding of European projects supporting those EU framework directives, that have used DPSIR as a central pillar in environmental problem-framing. As indicated above, it has been recognized as a valuable problem structuring method, both within scientific circles as well as its adoption by international organizations. It is perhaps less surprising that there is less use in fully marine systems than in coastal systems, where there are greater populations and environmental problems. In our comprehensive review, only 26 studies covered exclusively marine habitats and from these only eight illustrate concrete case studies. It is expected that in future more studies will focus on fully marine ecosystem due to the further implementation of the European Marine Strategy Framework Directive (2008/56/EC) and the European Marine Spatial Planning Directive (2014/89/EU).

DPSIR—Non-standard Use of Terms

The wide variety of derivatives is shown in their evolution over time in **Figure 1C**. Most of the frameworks derive directly from DPSIR after 1999, although the DPSEEA-eDPSEEA branch used primarily in health/medicine appeared to diverge earlier. There is some differentiation in use between social sciences and natural sciences, although theoretically DPSIR and close derivatives should cover both types of science. However, more emphasis may be on one or the other depending on the use, where natural scientists may have stronger emphasis on the pressure/state side and the social scientist may have greater emphasis on the impact/response/drivers side. This emphasizes the singular essence of using the DPSIR framework and derivatives in its holistic treatment bridging natural and socio-economic systems and in being a common framework applicable to human and environmental health.

The large number of derivatives indicates that use is wide-open to interpretation and our experience has shown that even specifically within DPSIR there is a high degree of variation in how the major components are interpreted or defined. It thus becomes necessary to define how it is used in every study otherwise there is great confusion in whether a component is ascribed to driver/pressure, pressure/state, or state/impact (Wolanski and Elliott, 2015; Scharin et al., 2016). Under the DPSIR framework (EEA, 1999), there has been longstanding variation in the interpretation and use of various components Drivers-Pressures-State change-Impact-Response, in particular in relation to the P, S, and I components. For example, the term "pressure" is commonly used interchangeably with "activity" or Driving force (Robinson et al., 2008). Similarly, state change and impact are both commonly used in the context of impacts on the environment (Eastwood et al., 2007) whereas impact also commonly refers to the impact on society brought about by a state change to the environment (Atkins et al., 2011). This issue is highlighted by Martins et al. (2012) who also noted variation in the use of indicators between studies (in a fisheries context) as a direct result of this misinterpretation. Whilst there are multiple matrices of the links between sectors, activities and pressures, this has not been carried through to the links between pressures and state changes, state changes and impacts and pressures and impacts, probably due to the large number and complexity of these interactions. Most importantly, the links have not been quantified but remain mostly at the conceptual level.

The recent developments within and between recent EU funded projects (see above), often through their common membership by participants, has helped to standardize definitions and component lists and has given a more rigid structure in starting from concepts and moving to assessments, even though they may have used different definitions.

DPSIR—Oversimplifies the Problems

It is emphasized that the concept of DPSIR is well-illustrated to be sound in that it presents a logical, stepwise chain of cause-effect-control events that describe the progression from identification of a problem to its management. However, its application requires a deeper understanding of the relationships between the different DPSIR components (Bell, 2012) before the concept can be effectively applied and its limitations need to be acknowledged. For example, P-S-I components are not mutually exclusive, despite being commonly treated as such. In particular, the P and S components are strongly linked in that Pressure, as the mechanism of change, causes a number of physical state changes that ultimately lead to biological change (hence the variation in the interpretation of that described by the P, S, and I components), or it can cause immediate biological change. The timescale over which this change occurs is variable and, in dose-response terms, can be chronic (subtle over long time periods) or acute (immediate). However, a discrete classification of pressures and state changes does not acknowledge this (Niemeijer and de Groot, 2008; Svarstad et al., 2008) and therefore overlooks an important part of the process leading to state change. Whilst activities are linked to both the D and P components, DPSIR in its current form does not categorically address activities or follow the pathway through pressure, state change, and impact, thus not adequately illustrating clear cause-effect relationships (Carr et al., 2007), which makes it difficult to pinpoint management actions. This problem has been overcome by the DAPSI(W)R(M) model (Wolanski and Elliott, 2015; Scharin et al., 2016), at the top of the "evolutionary tree" in **Figure 1C**, where these relationships are inherently contained with a good balance between natural and social aspects.

The DPSIR approach has to reflect the increasing knowledge of the complexity in the system. It is widely acknowledged that multiple activities occur simultaneously and create in-combination effects, that a single activity can give rise to multiple pressures (termed cumulative effects), that a pressure may not necessarily lead to a state change or impact, that a pressure associated with one activity may act differently to the same pressure associated with another activity and that the severity and the potential for state change may differ (Smith et al., 2016). Hence, it will be regarded as being oversimplified if DPSIR focuses on one-to-one relationships, disregarding the complex interactions between multiple pressures, activities, the environment, and society (Niemeijer and de Groot, 2008; Svarstad et al., 2008; Atkins et al., 2011; Tscherning et al., 2012). This can prevent early detection of state changes and impacts and therefore prevent timely, targeted management. Bell (2012) argued that targeted research was necessary to improve understanding of the S and I components of DPSIR (i.e., the state of the environment and its links to social and cultural drivers and impacts on society).

DPSIR—Solutions and Recommendations for the Way Ahead

The existing models appear to be adequate for depicting the relationships between drivers/pressures and the habitat/biological component that might be affected (or have its state changed) but may be inadequate in addressing state change, what it is or how it arises. The science behind assessments is advancing as new knowledge becomes available, but it still has to deal with ecosystems that are complex, and where pressure-effect relationships on ecosystem components and interrelationships between these components are not fully understood at the quantitative level. This complexity is further highlighted by the 4000+ potential regional seas sector-pressure-component "impact chains" identified from the ODEMM project with state change components only identified at the very highest level (Knights et al., 2015). Consequently, whilst DPSIR provides a strong and well-accepted concept, there is room for much more development in refining the concept, methodologies and applications.

Clarity of Terms in the DPSIR Framework

It has recently been concluded that the DPSIR approach and its terms have several anomalies and flaws which require it to be revised (Wolanski and Elliott, 2015; Burdon et al., 2015; Scharin et al., 2016). The main discussion is given elsewhere (see for example, Wolanski and Elliott, 2015; Scharin et al., 2016) but in brief, this contends that the terms require more accurate definition. Furthermore, the DPSIR framework does not categorically refer to the human activities which give rise to pressures. The most recent proposal to optimize the DPSIR framework for environmental management (DAPSI(W)R(M)) (pronounced "*dapsiworm*"), gives a more accurate and complete indication of the DPSIR framework (Wolanski and Elliott, 2015; Scharin et al., 2016, defined in **Figure 1B**). The original components of DPSIR, and their definitions, are retained but clarified by the inclusion of activities within the framework (**Figure 3**). The term **Driver** thus needs to refer to the basic human needs such as food, shelter, security, and goods. In order to obtain these, society carries out **Activities** (fishing, aggregate extraction, infrastructure building) which in turn create **Pressures** which are defined as the mechanisms whereby an Activity has an effect, either positive or negative. These effects, when on the natural system (the physico-chemical and ecological system) then need to be referred to as **State Changes** to separate them from State, a description of the characteristics at one time. These State changes thus encompass alterations to the substratum, the water column and their constituent biota.

Once these effects occur on the natural system then society is concerned that there will be a resulting change on human welfare and on the ecosystem services which ultimately produce societal benefits (Turner and Schaafsma, 2015). Hence, this **Impact** is on the human Welfare. Those Impacts on human Welfare and State Changes on the natural system then need to be addressed using **Responses**. As the EU Directives refer to these responses as **Measures** then we can use the final term as Responses (using Measures). Those measures then include economic and legal instruments, technological devices, remediation agents, and societal desires (Barnard and Elliott, 2015).

Expansion of DPSIR—Coping with Complexity

Although as indicated above, DPSIR cannot remain merely a very good concept dealing with a single driver/activity/pressure,

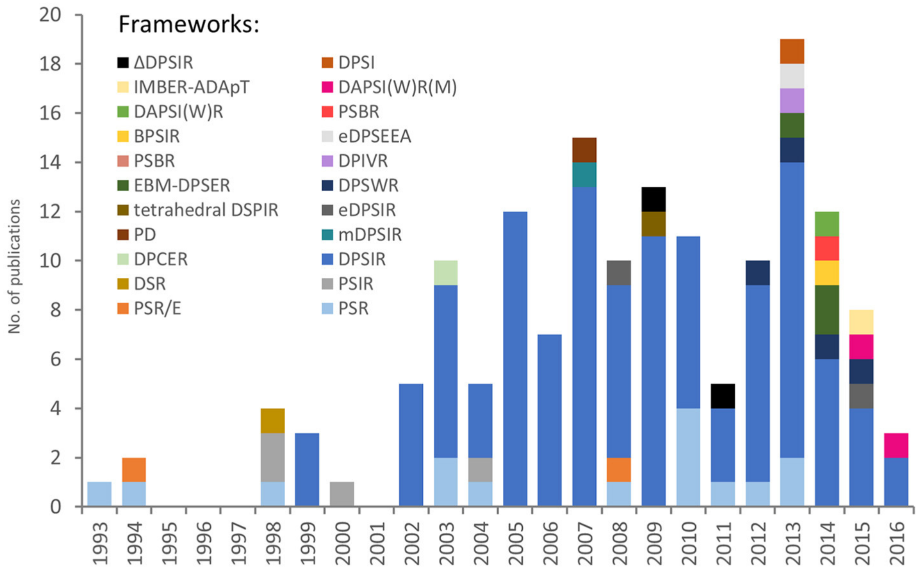

FIGURE 3 | Studies published per year (from those included in the review, $N = 152$).

given that ecosystems are rarely affected in this single mode and, from the point of view of effects on State Change, a single activity may cause more than one pressure (or mechanism of pressure) or multiple activities might cause a similar single pressure. Further difficulties may exist with different levels of the same pressure from different activities or differing activity spatial or temporal (timescale) footprints in a defined area. At another level, an impact from several pressures or activities might require a single or integrated response or measure. However, this has been recognized and there have been a number of developments to try and deal with more real-world and complex systems. Atkins et al. (2011) used the first nested-DPSIR approach where their marine case study area had many activities that required multiple DPSIRs nested to provide a more holistic view of complex ecosystems. The individual activity DPSIRs could be grouped with their Response components linked within one common Response area, which would comprise an integrated management plan of the case study area. Scharin et al. (2016) have also used this approach in a Baltic Sea case study with the more-evolved DAPSI(W)R(M) framework, where different sectoral activity chains each produce a state change where their sum total is the current state of the ecosystem. They also re-grouped the activities chains around Response to propose an integrated management plan. DAPSI(W)R(M) also can be nested spatially and sequentially, for example across ecosystem boundaries from a river catchment area through an estuary into the sea. Dolbeth et al. (2016) also used the Atkins et al. (2011) approach with nested DPSIR cycles grouped around a central management response, but with possible interactions between the different independent activity cycles and also beyond single area ecosystems at a pan-European level for lagoonal ecosystems. Smith et al. (2016) have also taken the Atkins et al. (2011) concept forward by rotating common grouped DPSIR cycles around a common pressure (for example seabed abrasion caused by individual DPSIR cycled marine activities) and then building up a three dimensional picture of an area affected by many different pressure cycles. All these developments have shown the adaptability of a simple DPSIR concept to a more complete ecosystem approach.

The essence of any framework which is to be successfully and widely applied is that it should be adaptable and, as emphasized here, have an ability to deal with generic and site-specific problems. It must encompass the inherent complexity and connectivity in all environments but especially marine, estuarine, and coastal systems. That adaptability resulting from complexity has been described by Gregory et al. (2013), using terms more common in social rather than natural sciences, as the need for the use of Problem Structuring Methods (PSMs) which enable us to learn from Complex Adaptive Systems (CAS) theory. In particular, both in general terms and specifically for marine environmental management this then encompasses and tackles what are regarded by social scientists as "wicked problems," a particular challenge in marine systems (Jentoft and Chuenpagdee, 2009; Gregory et al., 2013). While such "wicked problems" have been long-acknowledged in social sciences (Rittel and Webber, 1973), and regarded as problems that are "difficult or impossible to solve because of incomplete, contradictory, and changing requirements that are often difficult to recognize" (https://en.wikipedia.org/wiki/Wicked_problem), they are only now being acknowledged in the natural sciences. Here, we emphasize that we do now have the approaches, framework and background to tackle those problems.

Overall Approach

The analysis here has emphasized that, based on a long and extensive use, the DPSIR framework, its large number of derivatives and its recent expanded derivative DAPSI(W)R(M) has the potential as a holistic and valuable tool for analysing cause-effect-response links, determining management measures and communicating these aspects as long as it is used in its entirety. It is required to cover the complexity of coastal and marine systems, the competing and conflicting uses and users and their effects and management but in particular all steps from identifying the source of the problems, their causes and consequences and the means by which they are addressed. It has the potential as a visualization tool for complex interactions and so is valuable for the many stakeholders involved in managing the marine system.

The framework also has the flexibility to be applied across many systems and geographical, it can link marine systems and it can show the connectivity between adjacent systems. In particular, it shows the way in which environmental management is not only embracing complex systems analysis but is very well suited to it because of the many competing aspects. Similarly, to be effectively used it requires effectively merging natural and social science and cooperation between natural and social scientists and thus requires multi- and cross-disciplinary approaches. Hence, it has the ability to solve what may be the seemingly "wicked problem" of integrated marine assessment and management, but with the proviso that we need to keep moving from conceptual and generic models to those which are specific and quantified.

AUTHOR CONTRIBUTIONS

JP carried out the literature review, analysis, and prepared the figures. CS and KP set up the study and contributed data. All authors contributed to the text. All of the individuals entitled to authorship have been listed, contributed substantively to the research, read, and approved the submission of this manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmars.2016.00177

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Managing the Marine Environment, Conceptual Models and Assessment Considerations for the European Marine Strategy Framework Directive

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Conceptual models summarize, visualize and explain actual or predicted situations and how they might be tackled. In recent years, Pressure-State-Response (P-S-R) frameworks have been central to conceptualizing marine ecosystem issues and then translating those to stakeholders, environmental managers and researchers. Society is concerned about the risks to the natural and human system posed by those Pressures (thus needing risk assessment) and then needs to act to minimize or compensate those risks (as risk management). This research relates this to the DPSIR (Drivers-Pressure-State(change)-Impact-Response) hierarchical framework using standardized terminology/definitions and lists of impacting Activities and Pressures affecting ecosystem components, incorporating the European Marine Strategy Framework Directive (MSFD) legal decision components. This uses the example of fishing activity and the pressure of abrasion from trawling on the seabed and its effects on particular ecosystem components. The mechanisms of Pressure acting on State changes are highlighted here as an additional refinement to DPSIR. The approach moves from conceptual models to actual assessments including: assessment methodologies (interactive matrices, ecosystem modeling, Bayesian Belief Networks, Bow-tie approach, some assessment tools) data availability, confidence, scaling, cumulative effects and multiple simultaneous Pressures, which more often occur in multi-use and multi-user areas. In defining and describing the DPSIR Conceptual Framework we consider its use in real-world ecosystems affected by multiple pressures or multiple mechanisms of single pressures, and show how it facilitates management and assessment issues with particular relevance to the MSFD.

Keywords: DPSIR, risk, pressure mechanisms, exogenic pressures, endogenic pressures, assessment, benthic trawling

INTRODUCTION

Determining the cause and consequence of marine environmental problems entails risk assessment, and the responses entail risk management (Cormier et al., 2013). Conceptual models help to summarize, explain and address the identified risk by deconstructing each aspect being assessed, prioritized and addressed (Elliott, 2002). In risk management, these models communicate relevant knowledge to managers and developers as well as having an educational value (Mylopoulos, 1992), to increase awareness of the environmental risks through ocean literacy (Uyarra and Borja, 2016). This enables the development of quantitative and numerical models, hypothesis generation or for indicating the limitation of such models and the available scientific knowledge (Elliott, 2002).

Conceptual models are simple to complex diagrams which collate and summarize relevant information and so by their nature they may become increasingly complex, hence the term "horrendograms" (Elliott, 2002), but they are the pre-requisite for all numerical models.

A key current conceptual framework in widespread use, the Driver-Pressure-State-Impact-Response (DPSIR) framework (OECD, 1993), has developed over the last few decades and is used as the basis for many conceptual approaches addressing Pressure-State change links (Elliott, 2014; Gari et al., 2015). It structures and standardizes conceptualizing complex issues although at present it provides an overly simplistic representation of the relationship between Pressures and State changes, merely indicating that Pressure leads to State change (which may not necessarily be the case). It takes no account of the interaction between different Activities and their associated Pressures occurring simultaneously (Gari et al., 2015). Furthermore, it does not highlight the difference in the nature, severity, timescale or longevity of State changes in relation to pressure intensity, frequency or duration.

Today the DPSIR framework has produced many derivatives and refinements (e.g., Gari et al., 2015; Lewison et al., 2016) with the most extensive review undertaken by Patrício et al. (2016), covering some 152 studies and 27 major projects based around DPSIR, noting more than 23 derivative acronyms, with one further derivative recently being published (DAPSI(W)R(M)—Wolanski and Elliott (2015) and Scharin et al. (2016)). In this manuscript we use the terminology of the "DPSIR framework" rather than any one specific derivative, with emphasis on defining and clarifying components.

An improved understanding of the interactions between Drivers, Pressures and States (or, more particularly, the Pressure-State change (P-S) linkage) is important to help consider possible risk management responses. Pressures are the mechanisms that lead to State changes (and Impacts on human welfare). Hence a Pressure may be analogous to hazard as the cause of risk to an element. In turn, the risk is the probability of effect (likely consequences) causing a disaster or assets affected by the hazard (as human consequences) (Elliott et al., 2014). Smith and Petley (2009) consider that hazard, as a cause, and risk, as a likely consequence, relate especially to humans and their welfare. In the discussion here, the consequence may be regarded as relating to the Impact (on human Welfare) part of the DPSIR cycle (Cooper, 2013). Therefore, we can emphasize the links between the DPSIR approach and risk assessment and risk management.

European Union (EU) Member States must ensure no significant risks to, or impacts on marine biodiversity, marine ecosystems, human health or legitimate uses of the sea. This is enshrined in the Marine Strategy Framework Directive (MSFD; 2008/56/EC), an ambitious legislative instrument for the EU and indeed global marine environmental management which extends control of EU seas out to 200 nm (EC, 2008). Boyes and Elliott (2014) show its importance linking with other holistic and EU framework directives such as the Water Framework Directive (2000/60/EC), Habitats Directive (92/43/EEC), and the Maritime Spatial Planning Directive (2014/89/EU). The MSFD links the causes of marine environmental changes, human Activities and Pressures to their consequences leading to controlling and managing those causes and consequences. If successful, it will protect the natural system while also allowing the seas to produce ecosystem services and deliver societal benefits (Borja et al., 2013). The MSFD focuses on the assessment and monitoring of the functioning of marine ecosystems rather than just its structure. It aims to achieve Good Environmental Status (GES) by 2020 to ensure marine-related economic and social activities and via a roadmap for each Member State to develop an iterative strategy for its marine waters including assessments, determination of GES, establishment targets, indicators and monitoring with a programme of measures to achieve or maintain GES (EC, 2008, 2010; CSWP, 2011; CSWD, 2014). This structured approach allows each EU Member State to ensure there are no significant risks to marine ecosystems, human health or legitimate uses of the sea. Three Member States (Estonia, Denmark and Greece) used DPSIR in their MSFD initial assessments (CSWD, 2014), primarily in their socio-economic analyses.

This review focuses on the relevance of the DPSIR framework to the MSFD to organize and focus assessments in real marine situations including the linkages between multiple Activities exerting multiple Pressures and leading to State changes through multiple mechanisms (i.e., beyond simplistic single DPSIR chains). This ensures the DPSIR approach becomes more usable and a first choice starting approach to addressing marine issues. We standardize the approach incorporating ecosystem characteristics/components to allow ease of use in marine assessments, the movement from concepts to assessments and different assessment methodologies.

THE DPSIR FRAMEWORK

Rapport and Friend (1979) proposed the first Pressure-State-Response (PSR) framework which was then promoted by the Organization for Economic Cooperation and Development (OECD, 1993) for its environmental performance monitoring. This framework assumes causality that human Activities exert Pressures on the environment (marine and terrestrial), which can induce changes in the State/quality of natural resources. Society addresses these changes through environmental, governance, economic and sectoral responses (policies and programmes). Highlighting the cause-effect relationships can help decision makers and the public see how those issues are interconnected. The OECD (1993) re-evaluated the PSR model, whilst initiating work with environmental indicators. Its use has been extended widely and with many iterations (Patrício et al., 2016). The US Environmental Protection Agency (EPA, 1994) extended it to include the effects of changes in State on the environment (Pressure-State-Response/effects), UNEP (1994) further developed the Pressure-State-Impact-Response (PSIR) framework and the UN Commission on Sustainable Development proposed the Driving Force-State-Response framework (DSR). Here, Driving force replaced the term Pressure in order to accommodate more accurately the addition of social, economic and institutional indicators. Through agencies such as the European Environmental Agency and EUROSTAT, the EU adopted the Driving Force-Pressure-State-Impact-Response framework (DPSIR), as an overall mechanism for analyzing environmental problems (EC, 1999). The EU scheme (**Figure 1A**) shows that Driving forces (e.g., basic economic sectors) exert Pressures (e.g., carbon dioxide emissions), leading to changes in the State of the environment (e.g., changes in the physico-chemical and biological systems, nutrients, organic matter, etc.), which then lead to Impacts on humans and ecosystems (e.g., decreased fish production) that may in turn require a societal Response (e.g., research, building water treatment plants, energy taxes). The Response can feed back to Driving forces, Pressures, State or Impacts directly through adaptation or remedial action (e.g., policies, legislation, restrictions, etc.).

Interpretation of DPSIR has been variable and there has been the need to clarify terms which are often defined/used differently by natural and social scientists. For example, where either:

- **State** is the State of the Environment and **Impacts** are physical/chemical/biological changes to the state of the environment—natural science perspective, or
- **State** is State change (of the environment) and **Impacts** are the effects on human society and welfare—social science perspective

This lack of clarity has mostly led to further re-definition of one element of the model for example DPSWR where Impact has been replaced/clarified with Welfare (Cooper, 2013) or taking this further to DAPSI(W)R(M) [Driver-Activity-Pressure-State change-Impacts (on Welfare)-Responses (through Measures), (Wolanski and Elliott, 2015; Scharin et al., 2016)]. A clearer terminology (**Figure 1B**), is based on Borja et al. (2006), Robinson et al. (2008), and Atkins et al. (2011), for the DPSIR framework in natural ecosystems:

- ***Drivers:*** at the highest level, "Driving Forces" are the overarching economic and social policies of governments, and economic and social goals of those involved in industry. At a mid-level they may be considered to be Sectors in industry (e.g., fishing) and at a lower level, Activities in the Sector (e.g., demersal trawling).
- ***Pressure*** is considered as the mechanism through which an Activity has an actual or potential effect on any part of the ecosystem (e.g., for demersal trawling Activity, one Pressure would be abrasion to the seabed).
- ***State change*** refers to changes in the "State" of the natural environment which is effected by Pressures which cause State changes to ecological characteristics (environmental variables, habitats, species/groups structural or functional diversity) (e.g., abrasion may cause a decrease in macrofaunal diversity)
- ***Impacts*** are the effect of State changes on human health and society, sometimes referred to as Welfare, change in Welfare is affected by changes in use values and in non-use values (e.g., loss of goods and services from loss of biodiversity).
- ***Response*** is the societal response to Impacts through various policy measures, such as regulations, information, behavior change (e.g., ocean literacy), and taxes; these can be directed at any other part of the system (e.g., reduction in the number of bottom trawler licenses, the change to a less abrasive gear, or creation of no-fishing areas).

DPSIR CYCLES

Whilst a single DPSIR model or cycle (**Figure 1B**) greatly over-simplifies the "real world," it can conceptualize the relationships between environmental change, anthropogenic pressures and management options. However, to be of value, the model does need to be bounded (e.g., Svarstad et al., 2008), for example, by defining its spatial limits (usually the management unit such as a particular area of sea or length of coast). Furthermore, while a simple DPSIR cycle relates to the Activity or Sector to which it applies, the marine environment is a complex adaptive system (Gibbs and Cole, 2008) with areas subject to several Drivers. Accordingly, this requires to be visualized as several interlinked DPSIR cycles (each representing different interacting Activities or Sectors which compete for the available resources). Atkins et al. (2011) linked separate systems by the Response element, arguing that the effective management of anthropogenic impacts requires integrated actions (involving many types of response) affecting all relevant Activities; in contrast, Scharin et al. (2016) linked DAPSI(W)R(M) in similar cycles around State changes. Separate DPSIR cycles, each relating to a different Activity, can also be linked by Pressures and reflect the concept that several different Activities can create the same environmental pressure (**Figure 2A**). Following Atkins et al. (2011), **Figure 2A** illustrates how a single Pressure (the central blue circle) provides a common link between five separate DPSIR cycles, which represent five separate Activities. For clarity, the links within each individual DPSIR cycle have been simplified (e.g., by omitting the direct R-P link within each cycle and the links between other D, S, I, and R elements for different cycles *a la* Atkins et al., 2011). Linking separate DPSIR cycles in this way, and placing Pressure at the heart of the model, focuses attention on the Pressure as the system element that needs to be managed, thus supporting the assessment of Pressure-State change linkages. Hence, any such single Pressure may bring about a State change across a number of different ecological components. In essence, we assess State

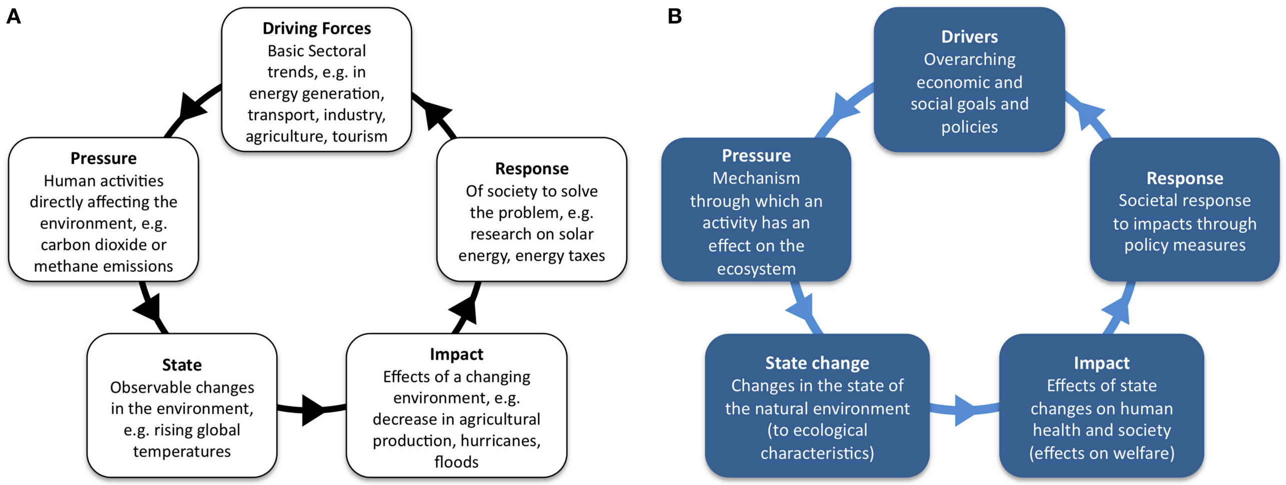

FIGURE 1 | Driving forces-Pressure-State-Impact-Response evolution. (A) DPSIR redrawn from the original EU framework (EC, 1999). **(B)** DPSIR as used by the authors. Reproduction is authorised provided the source is acknowledged.

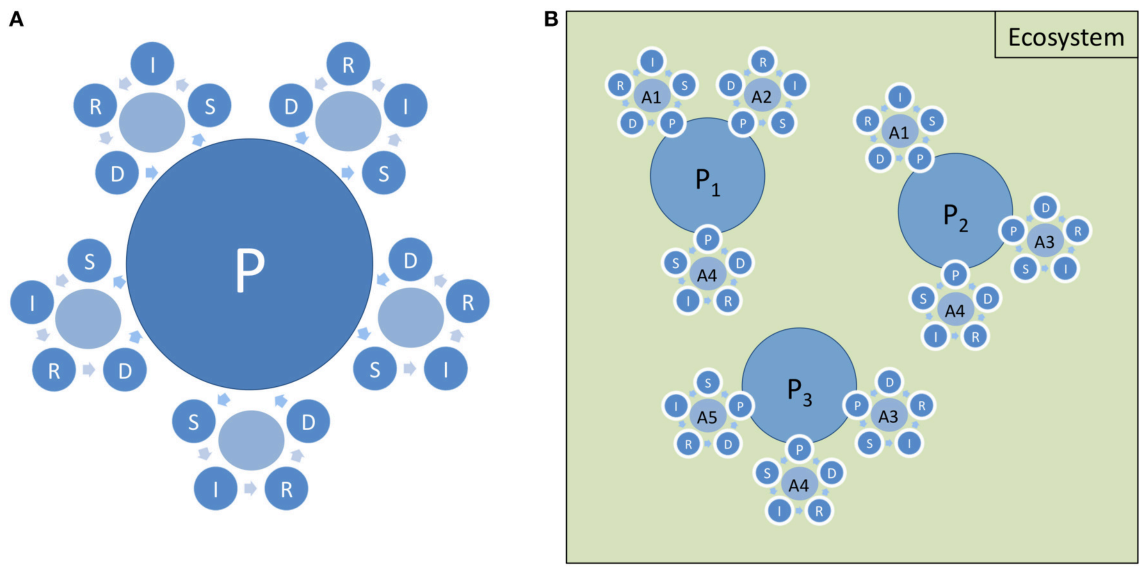

FIGURE 2 | Multispace DPSIR cycles. (A) Separate DPSIR cycles linked through a common Pressure element (e.g., abrasion pressure from the activities of benthic trawling, anchoring, dredging, etc.). **(B)** Example of linked DPSIR cycles in a particular ecosystem with individual separate Pressures (P1-P3), each associated with discrete Activity types (A1-A4). It is important to acknowledge that, just as the same Pressure may be generated by more than one Activity, so the same Activity may give rise to a number of different Pressures. For example, (P1) might represent abrasion, and may link DPSIR cycles relating to three Activities—benthic trawling (A1), anchoring (A2), and dredging (A3); (P2) might represent marine litter, linking benthic trawling (A1), the development of both non-renewable energy facilities (A4) and renewable energy facilities (A5); whilst (P3) might represent substratum loss, linking DPSIR cycles relating to the development of both non-renewable energy facilities (A4), renewable energy facilities (A5), and dredging (A3). For simplicity and clarity, Responses are shown here as having limited, within-cycle effects. In practice however, Responses within one DPSIR cycle may affect one or more of those other DPSIR cycles that are linked by a common Pressure or, indeed, DPSIR cycles that are not directly linked.

changes and Impacts but we manage the Drivers, the Activities and the Pressures, and in some cases State changes. Having a series of nested and linked DPSIR cycles, and linking these across ecosystems, accommodates many Pressures within one area (Atkins et al., 2011). Thus, a nested DPSIR cycle in a near-shore area, for example, has to link with those in the catchments, estuaries and at sea. This overcomes some of the difficulties in applying the framework to dynamic systems, cause-consequence relationships, multiple Drivers and only linear unidirectional causal chains.

It is necessary for the framework to accommodate multiple pressures and state changes which can lead to cumulative, synergistic or antagonistic impacts (Nõges et al., 2016; Teichert et al., 2016; **Figure 2A**). For example, the different cycles in

Figure 2B representing different Pressures or classes of Pressure, P1, P2, and P3 acting on an ecosystem [for example, (P1) might represent abrasion, and may link DPSIR cycles for three Activities—benthic trawling (A1), anchoring (A2), and dredging (A3); (P2) might represent marine litter, linking benthic trawling (A1), the development of non-renewable energy facilities (A4) and renewable energy facilities (A5); whilst (P3) might represent substratum loss, linking DPSIR cycles relating to the development of non-renewable energy facilities (A4), renewable energy facilities (A5), and dredging (A3)]. Hence there are many links between DPSIR chains across the different levels; for example, where the Responses and Drivers for one Activity interact with or affect the Responses and Drivers for a different Activity.

DPS CHAINS IN THE MSFD

The MSFD lists indicative characteristics, pressures and impacts to be taken into account during assessments (EC, 2008). There is some ambiguity in terms where the Directive presents "pressures" and "impacts" together, when pressures (P, Pressures in the DPSIR framework) should be distinguished from Activities, and Pressures should be distinguished from adverse effects on the natural system (i.e., S, State changes in the DPSIR framework). These lists have evolved since first publication, for example, in DIKE (2011) and CSWP (2011), but some ambiguities still remain.

Activities

In addition to clarifying the terminology, we also advocate alternative tables that list Activities and Pressures based on the work of a number of MSFD-related EU funded projects, particularly ODEMM (https://www.liverpool.ac.uk/odemm), VECTORS (www.marine-vectors.eu) and DEVOTES (www.devotes-project.eu). A list of possible and/or existing Activities is needed from which a subset can be extracted that may contribute to a greater number and/or more detrimental pressures for risk assessment and risk management and used to fulfill programmes for monitoring and response measures. **Table 1** shows a complete Activities list contributing to Pressures, refined from the ODEMM project (White et al., 2013) where Activities had been separated into Sector and sub-sectors. To avoid duplication with either Driver or Activity, we consider that the term "Sector" is unnecessary, meaning that only an Activity is required to produce Pressures. Overall 13 major Activities characterize the wide range of sea uses.

Pressures

The MSFD Pressures list (EC, 2008) identifies eight Pressure themes with 18 individual Pressures or mechanisms. Robinson et al. (2008) listed further Pressures, which were later updated by White et al. (2013). Except for Pressures from climate change, Pressures predominantly relate to anthropogenic Activity, also referred to as endogenic managed Pressures (Atkins et al., 2011; Elliott, 2011; Elliott et al., 2014), i.e., emanating from within the system to be managed. Exogenic unmanaged Pressures, in contrast, are from outside of the system and mostly relate to climate change, isostatic/eustatic change, or seismic activity. Elliott (2011) emphasizes that whereas the causes and consequences of endogenic managed Pressures are addressed within a management scheme for a marine area, only the consequences (as opposed to the causes) of exogenic unmanaged Pressures can be addressed at management scales; for example, the consequences of climate change can be addressed locally whereas the causes require global action.

As the MSFD only refers to an incomplete list of endogenic Pressures, we have revised both the MSFD and the White et al. (2013) lists to give 26 managed Pressures of which 18 were listed in the Directive (**Table 2**) and 7 are unmanaged Pressures (**Table 3**). The unmanaged Pressures allow climate change to be considered as it has been omitted in MSFD implementation and barely mentioned in the Directive (Elliott et al., 2015). The latter concluded that shifting baselines, resulting from climate change, need to be accommodated and revised during monitoring, environmental status assessment and in management actions (i.e., programmes of measures). The spatial and temporal variation in the response of the various biological components to climate change needs to be understood, as well as their ability (or lack of it) to adapt and reach equilibrium. Climate change may also exacerbate other Pressures and changes in the Descriptors (11 broad qualitative environmental descriptors for which GES must be assessed) for example the movement of non-indigenous species by increased shipping, but these effects may be indistinguishable from those arising from other anthropogenic Activities. Long-term, spatially extensive data sets will be needed to identify changes in ecological indicators. Although such data sets are not widely available for all Pressures, some efforts have been made to solve this gap. For example, for non-indigenous species, several databases hosting and sharing such information have been gathered in the European Alien Species Information Network (EASIN, http://easin.jrc.ec.europa.eu/) (Katsanevakis et al., 2015).

Using the DPS

As a major example of the complexity of interactions we consider just one Activity, extraction of living resources from benthic trawling and its multiple individual Pressures affecting the seafloor environment (see Blaber et al., 2000, and conceptual models in Gray and Elliott, 2009). In terms of Pressures, benthic trawling targets and results in the selective extraction of species but also brings about the non-selective extraction of other living resources and causes abrasion, scouring and turning over the sediment as well as causing compaction and other changes in the seabed. Fishing vessels can also input various objects/elements into the marine environment (e.g., noise, synthetic compounds, non-synthetic compounds, other substances, litter), and cause death by collision. Benthic trawling includes some 12 individual primary and lesser Pressures (**Table 2**) each with differing effects.

In turn, the trawling Pressures may be site-specific, acting on specific habitats and ecosystems; **Table 4** shows the European Commission MSFD-provided ecosystem components, with the first part highlighting habitats potentially impacted by benthic trawling—predominantly shallow to shelf sublittoral sedimentary habitats. The habitats in turn define and link with the potential

TABLE 1 | Activities contributing to Pressures (modified extensively from White et al., 2013).

Activity	Examples and concerns from the activity leading to pressures
Production of living resources	Aquaculture: fin-fish set-up and operations, macro-algae set-up and operation, shellfish set-up and operations, predator control, disease control, stock enhancement methods
Extraction of living resources	Benthic trawling, scallop dredging, fishery wastes, netting (e.g., fixed nets, seine netting), pelagic trawling, potting/creeling, suction hydraulic dredging, bait digging, seaweed and saltmarsh vegetation harvesting, bird eggs and shellfish hand collecting, peels, curios, recreational fishing, extraction of genetic resources
Transport	Litter and debris (unauthorized dumping), mooring/beaching/ launching, shipping, steaming, shipping wastes, passenger ferries, transport of goods, navigation, dredged material disposal
Renewable energy generation	Renewable (tide/wave/wind) power station construction and operations
Non-renewable energy generation	Fossil fuel (coal, oil, and gas) power stations, thermal discharge (cooling water), water abstraction, marine fracking, nuclear power, radioactive discharge and storage
Extraction of non-living resources	Inorganic mine and particulate waste, non-living maerl, rock/minerals (coastal quarrying), sand/gravel (aggregates), water for desalination, salt, navigational dredging, marine hydrocarbon extraction, capital dredging, maintenance dredging, substratum removal
Coastal and marine structure and infrastructure	Artificial reefs, barrages, beach replenishment, communication infrastructure (cables), constructions, culverting lagoons, dock/port facilities, groins, land claim, marinas, pipelines, removal of space and substrata, bathymetric/topographic change, sea walls/breakwaters, urban buildings, cables/pipelines/gas storage/carbon capture, cultural sites such as wrecks, foundations, sculptures
Land-based industry	Industrial effluent treatment and discharge, industrial/urban emissions (air), particulate waste, desalination effluent, sewage and thermal discharge, power plant discharges
Agriculture	Coastal farming, coastal forestry, agricultural wastes, land/waterfront run-off
Tourism/recreation	Angling, boating/yachting, diving/dive site, litter, littering/dumping, debris, bathing, public beach, tourist resort, water sports
Defense and national security	Military activities, hazardous material disposal areas, infrastructure (naval bases, ports, airports, degaussing stations), vessels, vehicles, sonars and munitions testing and use at sea, mooring/anchoring/beaching, dumping
Research and conservation	Animal sanctuaries, marine archeology, marine research, physical sampling, physico-chemical and biological sample removal
Carbon sequestration	Storage, exploration, construction, operational

biological components present (e.g., shallow sublittoral muddy sand supporting seagrass).

Within any one habitat, the different Pressures may affect several environmental characteristics (**Table 4**, highlighted) which also define/affect the niches of species groups (**Table 4**, highlighted) such that following a Pressure, the environmental characteristics may no longer be suitable for that species group. Each of those species groups has structural and functional characteristics (**Table 4**, highlighted) that may be affected to various extents. Although most of the effects that have been highlighted are direct, there are indirect effects for example through damage or habitat modification or changes to predator-prey relationships.

The situation is further complicated as different Pressure levels create different State change trajectories; for example, a Pressure causing large scale direct mortality will immediately reduce species, abundance, biomass, diversity, community structure, etc., and the duration of this depends on the nature of the habitat and its recovery potential (Duarte et al., 2015). The degree of Pressure then determines the severity and timescale of wider effects (e.g., at higher trophic levels) or on individuals (e.g., crushing, loss or damaged limbs or shells through collision with fishing gear) so that energy is allocated to individual recovery rather than growth/reproduction etc. In the long term, biomass, some components of population and community may be compromised with wider effects at the ecosystem level.

REFINING DPSIR PRESSURE-STATE CHANGE RELATIONSHIPS

Whilst it is well understood that Pressures on environmental systems can result in varying degrees of State change causing, for example, a loss of biodiversity and ecosystem services, the process by which those Impacts occur is complex. For a single, specific Pressure, the relationship between Pressure and Impact varies according to the degree of Pressure (e.g., spatial extent, duration and/or frequency, intensity), the habitat type upon which the Pressure is acting, the component species and those species in the wider ecosystem which they support. This produces many potential Pressure-State change trajectories that increase in complexity with concurrent potentially synergistic or antagonistic combinations of Activities and Pressures (Griffen et al., 2016). Hence the need to move from a conceptual framework to "nested horrendograms" to encompass the interlinked complexity (e.g., Elliott et al., 2015). Thus, generic processes leading to Impacts for a selection of Activities, Pressures, habitat types and biological components, then require specific, detailed trajectories that are site/system specific and specific to the nature of the Activities and their associated Pressures.

Current attempts to link Pressure with State change assume Pressure to act as a single mechanism leading to State change

TABLE 2 | Endogenic managed Pressures in the marine environment.

Pressure	Description
Smothering*	By man-made structures/disposal at sea
Substratum loss*	Sealing by permanent construction (coastal defenses/wind turbines), change in substratum due to loss of key physical/biological features, replacement of natural substratum by another type (e.g., sand/gravel to mud)
Changes in siltation and light regime*	Change in concentration of suspended solids in the water column (turbidity), deposition/accretion (dredging/run-off)
Abrasion*	Physical interaction of human activities with the seafloor/seabed flora and fauna causing physical damage (e.g., trawling)
Selective extraction of non-living resources*#	Aggregate extraction/removal of surface substrata, habitat removal
Noise*	Underwater noise—Shipping, acoustic surveys; surface noise (including esthetic disturbance)
Thermal regime change*	Temperature change (average, range, variability) due to thermal discharge (local)
Salinity regime change*	Freshwater—seawater balance, seabed seepage
Introduction of synthetic compounds*	Pesticides, antifoulants, pharmaceuticals, organohalogens
Introduction of non-synthetic compounds*	Heavy metals, hydrocarbons, PAH, organometals
Introduction of radionuclides*	Radioactivity contamination
Introduction of other substances*	Solids, liquids or gases not classed as synthetic/non-synthetic compounds or radionuclides
Nitrogen and phosphorus enrichment*	Input of nitrogen and phosphorus (e.g., fertilizer, sewage)
Litter*	Diffuse introduction of litter
Input of organic matter*	Input of organic matter (e.g., industrial/sewage effluent, agricultural run-off, aquaculture, discards, etc.)
Introduction of microbial pathogens*	Introduction of microbial pathogens
Introduction of non-indigenous species and translocations*	Through fishing activity/netting, aquaculture, shipping, waterways, loss of ice cover, genetic modification
Selective extraction of species*#	Removal and mortality of target (e.g., fishing) and non-target (e.g., by catch, cooling water intake) species
Aesthetic pollution	Visual disturbance, noise, and odor nuisance
Collision	Caused by contact between biological components and moving parts of a human activity (ships, propellers, wind turbines)
Barrier to species movement	Obstructions preventing natural movement of mobile species, weirs, barrages, causeways, wind turbines, etc. along migration routes
Emergence regime change (local)	Change in natural sea level (mean, variation, range) due to man-made structures
Water flow rate changes (local)	Change in currents (speed, direction, variability) due to man-made structures
pH changes (local)	Change in pH (mean, variation, range) due to run-off/change in freshwater flow, etc.
Electromagnetic changes	Change in the amount and/or distribution and/or periodicity of electromagnetic energy from electrical sources (e.g., underwater cables)
Change in wave exposure (local)	Change in size, number, distribution and/or periodicity of waves along a coast due to man-made structures

**Notes original pressure listed in the MSFD. #Whilst extraction is clearly an Activity, the specific extraction of non-living resources or species is considered here as a Pressure, as extraction is the mechanism of State change.*

TABLE 3 | Exogenic unmanaged Pressures in the marine environment (none originally or currently listed in the Marine Strategy Framework Directive).

Pressure	Description
Thermal regime change	Temperature change (average, range, variability) due to climate change (large scale)
Salinity regime change	Salinity change (average, range, variability) due to climatological events (large scale)
Emergence regime change	Change in natural sea level (mean, variation, range) due to climate change (large scale) and isostatic rebound
Water flow rate changes	Change in currents (speed, direction, variability) due to climate change (large scale)
pH changes	Change in pH (mean, variation, range) due to climate change (large scale), volcanic activity (local)
Change in wave exposure	Change in size, number, distribution and/or periodicity of waves along a coast due to climate change (large scale)
Geomorphological changes	Changes in seabed and coastline changes due to tectonic events

(Knights et al., 2011; Robinson and Knights, 2011; White et al., 2013). Hence, Pressure is the cause of physico-chemical and biological State changes which, through lethal or sub-lethal processes, compromise the performance or survival of one or more level of biological organization (cell, individual, population, community) (see **Figure 3**, overall organization). For example, the physical environment may be unsuitable to support the existing biological community, thus changing species

TABLE 4 | Marine Strategy Framework Directive (MSFD) components, highlighted for those impacted by benthic trawling.

Habitats (predominant habitats related to monitoring)	
• Littoral rock and biogenic reef	• Upper bathyal rock and biogenic reef
• Littoral sediment	• **Upper bathyal sediment**
• Shallow sublittoral rock and biogenic reef	• Lower bathyal rock and biogenic reef
• **Shallow sublittoral coarse sediment**	• Lower bathyal sediment
• **Shallow sublittoral sand**	• Abyssal rock and biogenic reef
• **Shallow sublittoral mud**	• Abyssal sediment
• **Shallow sublittoral mixed sediment**	• Reduced salinity water
• Shelf sublittoral rock and biogenic reef	• Variable salinity (estuarine) water
• **Shelf sublittoral coarse sediment**	• Marine water: coastal
• **Shelf sublittoral sand**	• Marine water: shelf
• **Shelf sublittoral mud**	• Marine water: oceanic
• **Shelf sublittoral mixed sediment**	• Ice-associated habitats
Environmental characteristics	
• Bathymetry	• Mixing characteristics
• **Topography**	• **Turbidity**
• **Sediment composition**	• Residence time
• Temperature	• Salinity
• Ice cover	• **Nutrients**
• Current velocity	• **Oxygen**
• Upwelling	• pH
• Wave exposure	• pCO_2
Species groups	
• Microbes	• **Fish**
• Phytoplankton	• **Cephalopods**
• Zooplankton	• Birds
• Angiosperms	• Reptiles
• Macroalgae	• Marine mammals
• **Benthic invertebrates**	
Structural characteristics	**Functional characteristics**
• **Species composition**	• **Functional diversity**
• **Species distribution/range**	• **Productivity**
• **Species variability**	• Fecundity
• **Abundance**	• **Survival**
• **Age/size structure**	• **Mortality**
• **Biomass and ratios**	• **Bioturbation**
• **Population dynamics and condition**	• **Predator-prey processes**
• Non-indigenous species	• **Energy flows**
• Chemical levels/contaminants	

Bold highlighted: strongly impacted by benthic trawling; light highlights indicates lesser influence by benthic trawling. Components adapted from (EC, 2008, 2010) and CSWP (2011, 2012). Benthic habitats: littoral (approximately 0–1 m – intertidal zone), shallow sub-littoral (approximately 1–60 m), shelf sub-littoral (approximately 60–200 m), upper bathyal (approximately 200–1100 m), lower bathyal (approximately 1100–2700 m), abyssal (approximately >2700 m).

composition and relative abundance (O'Neill and Ivanović, 2016).

Achieving State change can be a progressive process and whilst changes to the physico-chemical and biological structure may be classed as State changes, paradoxically they may also be viewed as the mechanisms through which a Pressure acts to cause a biological State change (i.e., not mutually exclusive as the DPSIR model suggests). For example, a substratum change during an Activity is a physico-chemical State change and at the same time is a mechanism (and hence a Pressure) resulting in a biological State change in the benthos (see examples on trawling impacts in Clark et al., 2016). Hence, whilst most Pressures are associated with physical State changes (e.g., hydrodynamic changes, substratum changes), the direct removal of species, the introduction of non-indigenous species and the input of microbial contaminants represent biological mechanisms of change.

These physico-chemical and biological modifications to the environment lead to a series of biological State changes, which can occur at any level of biological organization (Solan and Whiteley, 2016). Responses may be lethal (referring to loss) as a result of direct mortality associated with the Pressure, direct removal (e.g., by fishing gear) or emigration, or sublethal. Lethal responses can have immediate, direct effects on an individual, population and community (and ultimately ecosystem) in terms of the species composition, their relative abundance and biomass, total population and community biomass, trophic interactions and other functional attributes such as primary and secondary production and biogeochemical cycling. Sublethal responses relate to physical, chemical or biological damage caused by the Pressure at an individual level, whereby the organism survives but its performance and, therefore, contribution to ecosystem processes is compromised. Hence, biological State changes to the lower levels of organization (individual, population) will, if unchecked, lead to higher level (community, ecosystem) changes (Borja et al., 2015). The ultimate degree of State change at a community or ecosystem level associated with lethal and sub-lethal mechanisms of State change may be broadly similar but their severity, extent and duration will differ (Amiard-Triquet et al., 2015).

Despite this, the inherent variability and complexity throughout the levels of biological organization may mean that an effect at a lower level does not necessarily manifest itself at higher levels, i.e., stressors at lower levels (e.g., cellular, individual) may get absorbed so that the higher levels (e.g., population, community, ecosystem) do not show any deleterious ecological effects. The ability to absorb that stress has been termed environmental homeostasis (Elliott and Quintino, 2007).

The severity and sequence of biological State changes will vary according to:

- type and degree of Pressure (spatial extent, intensity, duration, frequency) and whether it leads to lethal or sub-lethal effects;
- habitat sensitivity and the potential for disturbance and recovery of the physical attributes;
- sensitivity of the component species and communities and their recovery potential (resilience);

- sensitivity of the balance of interactions within and between habitats and biological components.

Using abrasion from benthic trawling as a specific worked example (**Figure 3**, fine detail), and assuming a sublittoral sedimentary (mud/sand) habitat, there are several physical State changes that may arise and which may, in turn, lead to a series of biological State changes (O'Neill and Ivanović, 2016).

The physical State changes associated with abrasion can be divided into those that cause immediate biological State change at higher biological levels (population/community/ecosystem), for example, by direct mortality, and those that cause a progressive State change over an extended time period (Eigaard et al., 2016; O'Neill and Ivanović, 2016). This leads to two different trajectories of State change (lethal and sub-lethal), which act over different timescales and may ultimately differ in severity and longevity (Gilkinson et al., 2005) or require a different intensity of stressor.

With respect to sub-lethal effects, "abrasion" can lead to various sedimentary changes (**Figure 3**, Physico-chemical State Change box). Since the benthic inhabitants are intimately linked to the substratum (Snelgrove and Butman, 1994), such changes, if of sufficient severity or duration, will physically impair biological community structure and its long term survival, larval settlement and recruitment (Alexander et al., 1993). Similarly, the removal of species will affect a feedback loop whereby the organisms modify the sedimentary conditions through bioturbation, bioengineering, biodeposition, etc. (e.g., Gray and Elliott, 2009). Additionally, those organisms that are more mobile may simply relocate to other areas. Whilst sedimentary changes can lead to species loss, it also presents opportunities for colonization by new species leading to an overall change in community structure. Coupled with this may be a change in community function, if species are replaced by functionally different species (Koutsidi et al., 2016). Abundance, biomass and secondary production would be influenced (and perhaps species richness and diversity), which may impact on wider ecosystem processes (Hiddink et al., 2006; Queiros et al., 2006). Whilst this impact would be more gradual than in the second (lethal effects) scenario, and may be partly counteracted by colonization by new species, overall community structure and function may nevertheless be altered.

Additionally, sub-lethal effects may arise through (for example) morphological damage (caused by interaction with fishing gear) and the associated physiological stress, changes in the physico-chemical parameters of the water column (e.g., dissolved oxygen, suspended solids), clogging of respiratory structures, inability to feed or burrow and behavioral modifications (Tillin et al., 2006). Subsequently, somatic growth and reproductive capacity may be compromised as a result of, for example, increased respiration rate, increased ammonia production in response to stress, re-allocation of resources to survival and recovery (e.g., Widdows et al., 1981) or evolutionary adaptations that enable accelerated maturation and early reproduction at the expense of ultimate body size (Mollet et al., 2007; Elliott et al., 2012). These effects may

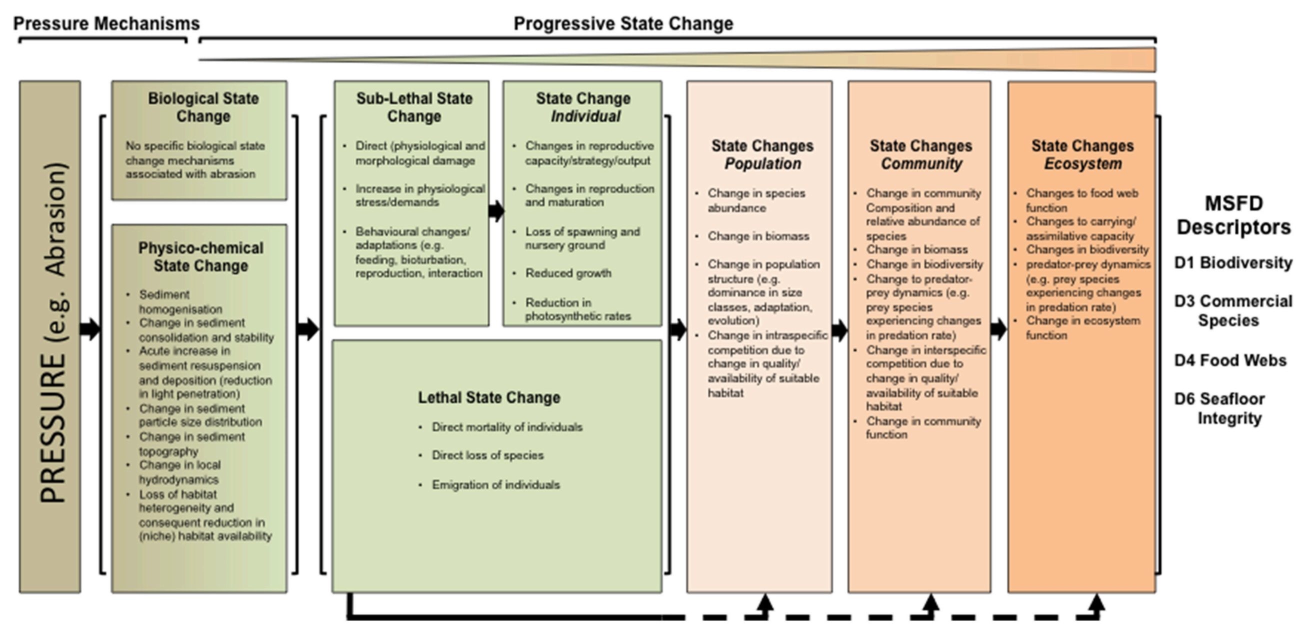

FIGURE 3 | Conceptual model showing the progression of physico-chemical and biological State changes arising from Pressures in the marine environment. The black arrows under the diagram indicate the way in which Pressure can cause a biological State change at any level: either (1) progressively through a sub-lethal response at the individual level which, over time, can lead to State changes at higher levels or (2) directly by acting at a higher level, leading to more immediate community and ecosystem State changes. Example details are given for the Pressure of abrasion from benthic trawling in a subtidal sedimentary habitat and links to the MSFD descriptors (e.g., through physico-chemical, structural or functional indicators at different levels from individual to ecosystem for descriptors D1 biological diversity, D3 commercial fish species, D4 food webs and D6 seafloor integrity).

initially be apparent at the individual or population level but, if sustained, will ultimately change abundance, biomass and function at community and ecosystem levels (Thrush et al., 2016).

Lethal effects will create immediate State changes at the population and community level, including biomass and abundance declines in both target and non-target species (Hiddink et al., 2006; Koutsidi et al., 2016). In the longer term, and particularly with frequent benthic trawling, a sustained reduction in species richness and diversity may occur, coupled with changes to community structure and function (Bremner et al., 2003). Population structure in disturbed habitats may also be altered, particularly in longer-lived species, as certain size classes are selectively removed or where species of a more opportunistic nature allocate resources to reproductive output rather than somatic production resulting in a population dominated by small and or/young individuals. Ultimately, these State changes will reduce secondary production which, coupled with altered predator-prey interactions, will alter higher ecosystem processes (Thrush et al., 2016).

In terms of timescale, and regarding the ability of MSFD indicators to detect State change, such sub-lethal population and community level changes are likely to be relatively acute (and rapidly detectable) processes. The duration would depend on the sensitivity of the species and habitats, their resilience (or their potential to recover to an alternative state which supports wider ecosystem processes) and the intensity of the Pressure (or causative Activity). It also depends on the processes in the first (sub-lethal) scenario, since the two do not occur in isolation, whereby physical and biological changes to the environment will influence recovery rates and trajectories (Foden et al., 2010; Lambert et al., 2014).

The above changes in these scenarios (lethal and sub-lethal) have the potential to ultimately produce overall negative effects at higher trophic levels and wider ecosystem processes. The difference between the scenarios lies in the complexity/detail trajectory between the application of a Pressure and the resultant State change. Finally, the effects of trawling can result in human welfare being affected through the reduction in the provision of ecosystem services (Muntadas et al., 2015) and societal benefits (Atkins et al., 2011). The resulting changes compromise the performance or survival of an ecological component and so may bring about State change detected by MSFD descriptors [e.g., at the population, community or ecosystem level for descriptors D1 (biological diversity), D3 (commercial fish species), D4 (food webs) and D6 (seafloor integrity)].

Whilst the scenario above relates only to a single Pressure, abrasion, this Pressure may potentially arise as the result of a number of different Activities (**Table 5**).

ISSUES IN MOVING FROM CONCEPTS TO ASSESSMENTS

Environmental management issues involve many challenges in moving from a conceptual framework to a data-based or expert judgment-based assessment. This involves identifying all the components and their linkages (e.g., D-P-S chains), and data/indicators and their quality or thresholds, etc.

Regional Seas

The European regional seas cover approximately 11,220,000 km^2 (EEA, 2014) with a wide range of environmental conditions and different ecosystems, which vary in diversity and sensitivity. This affects the repercussions of human Activities and their resultant Pressures. Pressures in one regional area may not have the same footprint (type, extent, or duration) in another area because of differing conditions (see examples in the Baltic Andersen et al., 2015, Mediterranean Claudet and Fraschetti, 2010, and Black Seas Micheli et al., 2013). For example, the Mediterranean Sea is characterized by high salinity, high temperature, predominantly wind-driven or water mass difference-driven currents, deep water, oligotrophic conditions with a fauna exhibiting low abundance and biomass. In contrast northern waters have opposing characteristics where, for example, tidally-driven mixing may create a different footprint of a Pressure (Andersen et al., 2013). The regional seas also have contrasting developmental and socio-economic issues producing complex and fragmented governance systems (Raakjaer et al., 2014). Although each of the regional seas have their own conventions (North-East Atlantic, Oslo/Paris Convention; Baltic Sea, Helsinki Convention; Mediterranean Sea, Barcelona Convention; Black Sea, Bucharest Convention) with similar objectives and targets, there are differences in the cohesiveness of each regional seas EU Member States and state of developed/stability of the related bordered countries. Geographically differing stages of development influence the status, quality and quantity of monitoring programmes producing data for assessments of Drivers, Pressures and State change (Patrício et al., 2014).

Data Availability

Within a causal link framework and to provide the route for and efficacy of management, indicators and their component indices/metrics are needed to determine the level of Pressure, and changes in State and Impact (e.g., Aubry and Elliott, 2006). The trajectory of State change can be used to determine targets or reference conditions for the assessment of the indicators (see Borja et al., 2012) which requires developing assessment methods or indices such as those within the Water Framework Directive (Birk et al., 2012). However, they need to be validated and calibrated against independent abiotic datasets (Birk et al., 2013). As some of the MSFD descriptors are related to Pressures, whilst others are related to State change, data analysis is needed to assess the effects that Activities have on marine physical, chemical and biological quality. Consequently all the relevant Activities, Pressures, States and their indicators need to be identified together with the linkages (cause-effect interactions) between them. The ODEMM Project linkage framework (Knights et al., 2013; White et al., 2013), for example, provides a means to fully evaluate all components that can affect the achievement of GES in a fully integrated ecosystem assessment. Applying a framework relies on having not only indices of change but also baselines, thresholds and targets against which to judge that change. In addition, there is the need to define the inherent

TABLE 5 | Activities (related to Table 1) that may give rise to abrasion Pressures on the seabed.

Activity	Sub-activity
Production of living resources	Set-up of fin-fish aquaculture facilities (interaction with seafloor during set-up of infrastructure, loss of gear)
	Operation of fin-fish aquaculture facilities (waste products, anti-fouling, predator control, disease and disease control, infrastructure effects on local hydrography, escapees, litter, anchoring/mooring of boats)
	Set-up of macro-algae aquaculture facilities (trampling (certain species), interaction with seafloor, removal of habitat-structuring species, loss of gear)
	Operation of macro-algae aquaculture facilities (waste products, anti-fouling, predator control, disease and disease control, infrastructure effects on local hydrography, litter, anchoring/mooring of boats)
	Set-up of shellfish aquaculture (interaction with seafloor when dredging for brood stock, loss of gear, litter)
	Operation of shellfish aquaculture (waste products, anti-fouling, predator control, disease and disease control, infrastructure effects on local hydrography, litter, anchoring/mooring of boats)
Extraction of living resources	Operation of benthic trawls and dredges—fishing (interaction with seafloor)
	Operation of benthic trawls and dredges—mooring/anchoring (interaction with seafloor)
	Operation of suction/hydraulic dredges (interaction with seafloor, catch, bycatch, waste products)
	Operation of suction/hydraulic dredges—mooring/anchoring (interaction with seafloor)
	Bait digging—(trampling, interaction with seafloor, removal of habitat-structuring species)
	Seaweed and saltmarsh vegetation harvesting (trampling, interaction with seafloor, removal of habitat-structuring species)
	Bird egg collection—(trampling, removal of individuals)
	Shellfish hand collecting—(trampling, interaction with seafloor, removal of individuals)
	Collection of peels/peeler crabs (boulder turning)—(trampling, removal of individuals)
	Collection of curios—(trampling)
Transport	Mooring/anchoring/beaching/launching (interaction with seafloor)
Renewable energy generation	Construction of wind farms (installation/deinstallation of turbines on seafloor includes interaction with seafloor, habitat change and sealing, laying cables)
	Construction of wave energy installations (cable laying/removing—localized habitat change, noise)
	Construction of tidal sluices (interaction with seafloor, localized sealing of habitat)
	Construction of tidal barrages (interaction with seafloor, habitat change (upstream and downstream) and localized sealing of habitat, barrier to movement for migratory anadromous or catadromous species)
Non-renewable energy generation	Exploration/construction of oil and gas facilities (drilling, anchoring, construction of wellheads, laying pipelines, oil spills) and subsequent decommissioning (anchoring, oil spills, removal of infrastructure where relevant)
	Construction of (land-based, coastal) power stations (jetties and intake wells—habitat change, sealing, increased turbidity, noise)
	Construction of (land-based, coastal) nuclear power stations (jetties and intake wells—habitat change, sealing, increased turbidity, noise)
Extraction of non-living resources	Maerl extraction—removal of substrate (habitat change, interaction with seafloor, removal of habitat-structuring species)
	Coastal rock/mineral quarrying—extraction of substrate (habitat change, interaction with seafloor, contaminant release)
	Sand/gravel aggregate extraction—removal of substrate (habitat change, interaction with seafloor, contaminant release)
	Capital dredging—extraction of substrate (habitat change, interaction with seafloor, contaminant release, increased turbidity, noise)
	Maintenance dredging and associated extraction of substrate (habitat change, interaction with seafloor, contaminant release, increased turbidity, noise)
Coastal and marine structure and Infrastructure	Construction of artificial reefs (interaction with seafloor, habitat change)
	Construction of culverted lagoons (interaction with seafloor, habitat change, smothering, increased turbidity, noise)
	Construction of marinas and dock/port facilities (habitat change, sealing, interaction with seafloor, smothering, increased turbidity, noise)

(Continued)

TABLE 5 | Continued

Activity	Sub-activity
	Operation of marinas and dock/port facilities (anti-fouling, contaminants, interaction with seafloor from anchoring, litter) Construction of land claim projects (habitat change, smothering, increased turbidity, noise) Construction of coastal defenses—sea walls/breakwaters/groins etc. (habitat change, sealing, interaction with seafloor, smothering, increased turbidity, noise)
Tourism/recreation	Angling [catch, bycatch, interaction with seafloor (gear, and anchors if offshore)] Boating/Yachting/Diving/Water sports—mooring/anchoring/beaching/launching (interaction with seafloor) Public use of beach—general (trampling, litter) Construction of tourist Resort (habitat change, sealing, smothering, increased turbidity, noise)
Defense and national security	Military activity—mooring/anchoring/beaching/launching/dumping, munitions, infrastructures (interaction with seafloor)
Research and conservation	Research operations (specific to activity but can include: interaction with seafloor, catch, bycatch)
Carbon sequestration	Exploration, construction

variability ("noise") against which the "signal" of change is measured (Kennish and Elliott, 2011). Each of these requires a fit-for-purpose data background for each biological and physico-chemical component relevant to a particular stressor. Given that for many Activities, the amount of Pressure required to produce a given State change and thus Impact on human welfare is unknown, then the amount of data required to determine and assess the State change is also unknown. Furthermore, although this could be determined through power analysis, it cannot be used unless the inherent variability in the components is known. Hence, it is likely that the approaches advocated here will continue to be semi-qualitative at best and reliant on expert judgment (see below).

Cumulative/In-Combination Effects

As single Activities exert multiple Pressures and the marine ecosystem usually supports multiple Activities, we need to consider cumulative/co-occurring (within an Activity) and in-combination (between Activities) effects. The multiple Pressures will rarely be equal and will lead to cumulative and in-combination effects which may be synergistic or antagonistic (Griffen et al., 2016). To indicate some of difficulties in assessing cumulative impacts, Crain et al. (2008) analyzed 171 multiple stressors studies in marine and coastal environments and found effects to be 26% additive, 36% synergistic, and 38% antagonistic, while interaction type varied by response level, trophic level, and specific stressors. In another meta-analysis of 112 experimental studies Darling and Côté (2008) found similar combined effects of two stressors with 23% additive, 35% synergistic and 42% antagonistic. Despite the lack of knowledge at the community and ecosystem level elucidating or predicting effects of combinations of individual Pressure impacts, we can measure the status of an ecosystem that is impacted by multiple Pressures (Griffen et al., 2016; Nõges et al., 2016; Teichert et al., 2016). Hence, while we can identify some elements, we are unclear regarding the precise changes at a sub-species, species, population or community level.

Co-occurring multiple Activity/Pressure impacts, as cumulative and in-combination threats or impacts, have been investigated according to the footprints of a particular Driver/Activity and their overlap with habitats using spatial mapping/modeling (Nõges et al., 2016). Cumulative impacts (including both overlap and weighted cumulative methods) have been investigated at a global level by Halpern et al. (2008) producing global impacts maps but also at the European level, for example in the Baltic (Korpinen et al., 2013; Andersen et al., 2015), eastern North Sea (Andersen et al., 2013) and the Mediterranean-Black Seas (Claudet and Fraschetti, 2010; Coll et al., 2011; Micheli et al., 2013). These techniques may not be of direct use in assessing State changes, but may nevertheless be of value in spatial planning applications, for example, in identifying areas where high levels of protection may be necessary. It should be noted that an Activity does not always have to lead to an impact especially if mitigation measures are employed.

Assessment Scales and Scaling Up to Regional Seas

The connections between ecosystem features and human Activities (and their related Pressures) should determine the appropriate scale at which the ecosystem approach should be implemented (Borja et al., 2016). Defining these scales and their boundaries is imperative for any ecosystem-based management (EEA, 2014). For a well monitored small bay, a comprehensive assessment can be normally made, because the Drivers, Pressures, and State changes could be well understood, mapped and assessed. However, at a larger scale, not all issues may be well known; some areas have quantitative data, some have no data, and a more widespread range of very differing habitats may be included. Borja et al. (2013) suggest that the fundamental challenge of obtaining a regional quality status is by either having a broad approach and omitting or down-weighting point-source problems or summing the point-source problems (which may cover only a very small area) to indicate the quality status of the whole area. State change becomes much more complicated and diverse. An important issue is the mismatch between the quantitative information from the pressures and different descriptors and biodiversity components at a large scale (i.e., regional or sub-regional sea), making difficult the large-scale assessment of the response of indicators of change. During the first phase of MSFD implementation, the baseline assessment of the EU marine area in 2012 gave a very broad understanding of Pressures and impacts from human Activities. Although most Member States have reported on most descriptors, providing an overview of their marine environment, the quality of reporting varies widely between countries, and even within individual Member States, from one descriptor to another (EC, 2014; Palialexis et al., 2014). In addition, when different countries are involved in the assessment, the relevant information may come from many different sources, which each have their own assessment timescales, aims, indicators, criteria, targets and baseline values thus limiting not only direct comparison, but also coherence in implementation (Cavallo et al., 2016).

Levels of Confidence

A conceptual framework such as DPSIR aims to encompass all key components and interactions of an ecosystem problem. However, when moving to the next step of assessment, incorporating many types of data, confidence in the outcome becomes an issue for both the assessors and the users of the assessment. The level of confidence in an assessment depends on the degree of uncertainty associated with the method of assessment, data availability and adequacy, and knowledge and understanding. This requires distinguishing between the lack of knowledge and natural variability (Hoffman and Hammonds, 1994), and uncertainty in the future forecasted state (due to lack of long-term data sets and historical data and/or spatio-temporal variability of a biological indicator) as well as in the resulting ecosystem state post-management action, present challenges in target setting (Knights et al., 2014). Uncertainty is mostly addressed through monitoring programmes that have adequate spatio-temporal coverage (Borja et al., 2010), although the absence of reference conditions or clear targets makes it difficult to establish an accurate assessment (Borja et al., 2012). However, confidence can also be given through a range of methods from cumulative qualitative assessment of each metric and, for example, a traffic-light overall confidence assessment to a separate quantitative confidence metric (e.g., Andersen et al., 2010; Carstensen and Lindegarth, 2016). Despite this, most uncertainty is due to poor definition in the determination of deviation from that expected, in a physico-chemical or biological component. If the agreed targets against which indices and metrics are judged are not sufficiently well defined, then it is not possible to judge the efficacy of management measures.

MOVING TO THE NEXT STEP: ASSESSMENT

The intricacy and complexity of Driver-Pressure interactions, and the relationship of Pressures to State changes makes it difficult to undertake high level or quantitative assessments for management purposes. It requires knowledge of all the potential causal chains and State changes. The possible methodologies are broadly either a matrices approach or as a form of ecosystem modeling but the assessment is only as good as the knowledge and detail applied (Borja et al., 2016).

Simple Matrices Approach

Matrices are simple tables where Drivers (or, more specifically, the Activities resulting from them) can be related to Pressures, and where Pressures can be related to ecosystem components. These allow the identification of chains formed by particular causal links and permit linear analysis of the impact chain (Knights et al., 2013). The matrices record relationships between Activity and Pressures, and between Pressures and ecosystem components. The relationships that are represented are complex with, for example, any single Activity potentially causing many Pressures, and any single Pressure being caused by more than one Activity (i.e., a many-to-many relationship). The matrices can be linked simply by an overlap (Pressure X affects component Y) or through more detailed information on potential levels of interaction, for example showing high/low or increasing/decreasing changes to a component. The degree of State change caused by a Pressure on a habitat can be assessed in terms of: Activity area or footprint, frequency, persistence, and characteristics of the habitat/ecosystem component impacted, including sensitivity and resilience (ability for recovery) (Knights et al., 2015). Matrices and Pressure assessment approaches were used extensively in the ODEMM project (Knights et al., 2011; Robinson and Knights, 2011; White et al., 2013) and in the DEVOTES project (Barnard et al., 2015). They have also been used as standard tools for Pressure assessments by, for example, HELCOM and OSPAR (Johnson, 2008). Complex matrices and linkages can be compiled through databases where programming can be used to analyze and filter data, for example, to highlight Activities that need to be managed or sensitive ecological components that might be at risk of State change [e.g., the PRISM and PISA Access database tools developed through the U.K. Net Gain project (Net Gain, 2011)]. The accuracy and value of the matrix approach depends on identifying and parameterizing components and linkages for a particular area. They are valuable for assessments, and depending on how comprehensive they are, will show impacted components which may then allow prediction of the State changes under given circumstances.

Ecosystem Models

With the move toward ecosystem-based management, much attention has been devoted to ecosystem modeling. These models may be conceptual, deterministic (in which there is underlying theory or embedded mathematical relationships) or empirical in which the links are described statistically even when there is no apparent underlying theory. Some relate to the management of particular aspects of the ecosystem (e.g., Robinson and Frid, 2003; Plagányi, 2007) whilst other, more recent, models concern the whole natural ecosystem or socio-ecological system (i.e., “end-to-end” models) model development/application (e.g., Rose et al., 2010; Heath, 2012). Piroddi et al. (2015), in reviewing whole ecosystem models with respect to MSFD assessments, note that they are more relevant as they may better represent interactions with biodiversity components, for example, ECOPATH with ECOSIM, ATLANTIS or coupled lower trophic and high trophic models (Rose et al., 2010). The ability to apply models to Drivers and Pressure effects relies on knowledge of Activities/Pressures and being able to parameterize the elements accordingly. For example, if trawling causes a 30% reduction in suspension feeders in a modeled area, this figure can be applied to that biological component (according to temporal or spatial scales) (Petihakis et al., 2007). A specific model may not have the resolution to apply a precise mechanism or be applied at individual habitat scale. Whilst pelagic habitats may be defined by salinity, temperature, depth, nutrients, oxygen, etc., benthic habitats as different spatial entities (an important setting for all species groups) are generally not parameterized in models. Nevertheless, such models may well be able to accommodate indirect effects such as changes in predator-prey relations or be used in a predictive manner where climate change could be de-coupled from anthropogenic impacts.

Bayesian Belief Networks

Bayesian Belief Networks (BBNs; also referred to as belief networks, causal nets, causal probabilistic networks, probabilistic cause effect models, and graphical probability networks) offer a pragmatic and scientifically credible approach to modeling complex ecological systems and problems, where substantial uncertainties exist. A BBN is a graphical and probabilistic representation of causal and statistical relationships across a set of variables (McCann et al., 2006). It consists of graphically represented causal relationships (for example, the DPSIR D-P-S chain links) comprised of nodes that represent component variables and causal dependencies or links based on an understanding of underlying processes/relationships/association. Each node is associated with a function that gives the probability of the variable dependent on the upstream/parent nodes. As each variable is set with best data available and can include expert opinion, simulation results or observed data, this is flexible and also allows the information to be easily updated with improved data (from Hamilton et al., 2005; Pollino et al., 2007). Notwithstanding their potential, BBNs represent a relatively new modeling approach. They have only been applied to marine assessments in a limited way (e.g., Langmead et al., 2007; Stelzenmuller et al., 2015; Uusitalo et al., 2015). However, BBNs are becoming an increasingly popular modeling tool, particularly in ecology and environmental management. This is largely because they can be used in a predictive capacity and also, because they use probabilities to quantify relationships between model variables, while explicitly allowing uncertainty and variability to be accommodated in model predictions (Barnard and Boyes, 2013). They show high promise in adaptive management being iterative and especially in being able to combine both empirical data and expert knowledge, a necessary feature given the often poor data for those empirical relationships.

The Bow-Tie Approach

The Bow-tie method was initially presented as a conceptual model; whilst its original application was mostly in relation to industrial risk assessment and management (de Ruijter and Guldenmund, 2016), it is now increasingly being used in a qualitatively manner to explore the natural and anthropogenic causes of change, and the associated consequences and responses (e.g., Cormier et al., 2013; Smyth and Elliott, 2014; Burdon et al., in press). It facilitates analysis or assessment of a defined problem by focusing attention onto the areas of a system where the consequences of a potentially damaging event can be proactively managed. The Bow-tie method provides for a graphical representation of the expansion of the initial DPSIR environmental cause-and-effect pathway (Cormier et al., 2013). More specifically, it can be used to focus on the pathway between Pressure and State change, and provides a means of identifying where controls can be put in place either to control the occurrence of a particular event, or to mitigate for the effects of the event should it occur (see **Figure 4**). It comprises several components:

- The start of any Bow-tie is the identification of a "Hazard"—which is defined as a part of the system under consideration that has the potential to cause damage (e.g., benthic trawling).
- A Top Event is identified, representing the point where control would be lost over the Hazard. The Top Event is defined so as to be occurring just before events start causing actual damage.
- There are usually a number of "Threats" that might give rise to a given Top Event; if these threats are not prevented from occurring, or are not mitigated in some way, the realization of the Top Event could then cause a set of one or more "Consequences." There are usually several or many Threats and Consequences for every Top Event.
- The final stage of building a basic Bow-tie model is to identify potential barriers which can be placed either between the Threat and the Top Event as prevention measures, or alternatively as recovery, mitigation or compensation mechanisms that either prevent the Top Event from escalating into actual Consequences, or reduce the severity of the Consequences. Preventative measures can take several forms, including economic, governance, societal, political or technological devices, based on the 10-tenets of adaptive management and sustainability (Barnard and Elliott, 2015).

There may be several top events in any one area as the result of the Drivers and hazards such that nested Bow-ties are required in any assessment of cumulative impacts (Cormier, 2015). Similarly, the consequence of the loss of control in one Bow-tie sequence may become the top event in another (Smyth and Elliott, 2014). For example, the threat of the introduction of non-indigenous species may be a top event, the consequence of which may be that an area fails GES under the MSFD. In turn, the failure to meet GES will then become the Top Event which has legal and financial consequences, each requiring mitigation (Smyth and Elliott, 2014). It is of note that ICES (2014) has recommended that the Bow-tie framework be used to address cumulative and in-combination Pressures and their consequences.

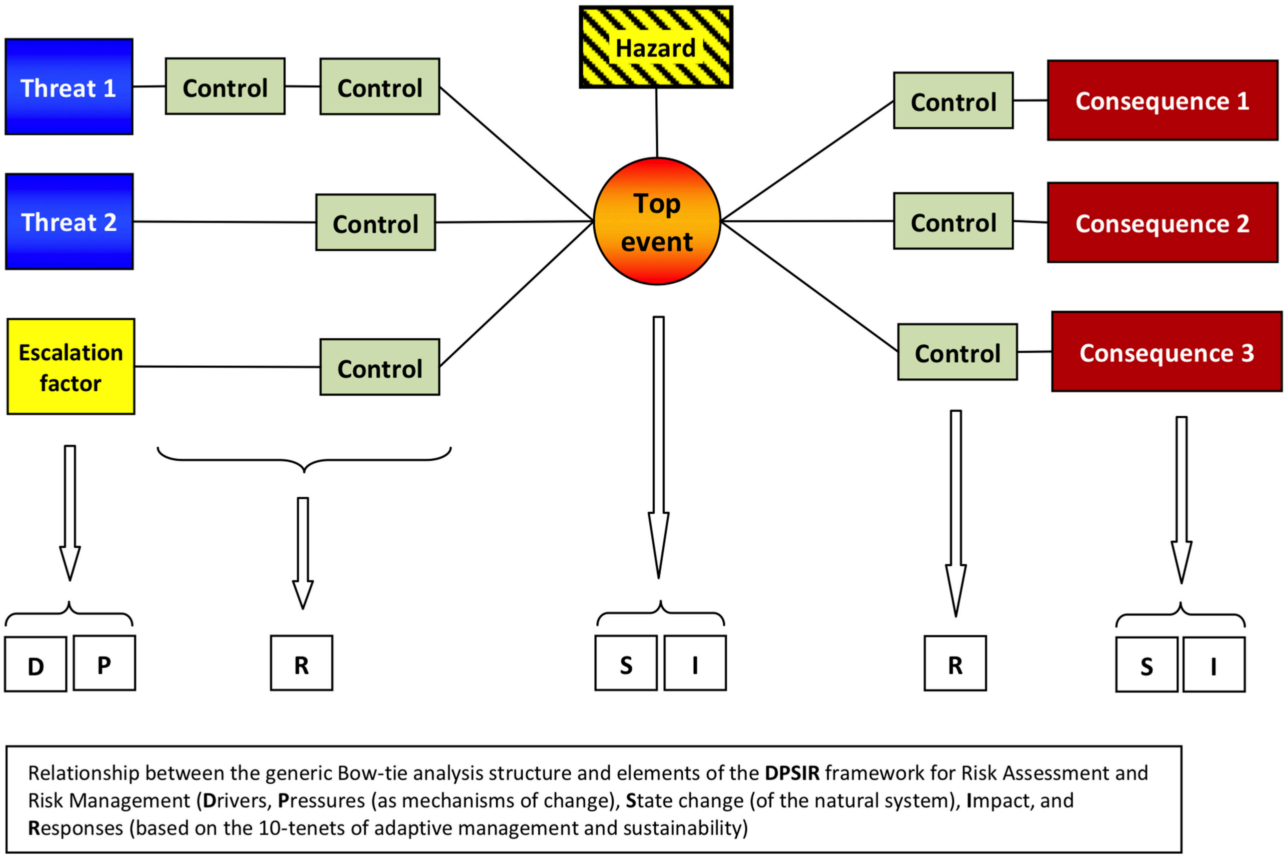

FIGURE 4 | Example of a Bow-tie structure and how it relates to DPSIR. This shows the Threats (left) and Consequences (right) relating to the Top Event (e.g., loss/decrease in marine wind resource/production ability, lower center) that is associated with a Hazard (environmental change due to climate change impacts, upper center). It also details the related Prevention Measures (left of center), Mitigation Measures (right of center), and Escalation Factors.

The DPSIR framework can be superimposed on the Bow-tie structure given that the threats to the top-event will be Drivers and/or Pressures and the top-event and consequences are likely to be the State changes and/or Impacts (**Figure 4**). The barriers both as prevention measures and as mitigation or compensation measures, constitute the Response within DPSIR. As such, this links to a risk assessment and then risk management (RARM) framework as the need for responses to human Pressures. Burdon et al. (in press) have directly linked the DAPSI(W)R(M) concept with Bow-tie in integrating natural and social sciences in a case study for the management of the Dogger Bank in the North Sea.

Nested Environmental Status Assessment Tool

The Nested Environmental status Assessment Tool (NEAT) is a recent tool for biodiversity assessments based on State indicators (Borja et al., 2016; and see therein for older, similar assessment tools). NEAT is a specialized user-friendly desktop application developed recently within the EU DEVOTES project (Berg et al., 2016) specifically targeted toward MSFD biodiversity assessments for defined spatial areas. It does not relate to Activities or specific Pressures, rather levels of State in relation to targets/thresholds. Assessments are indicator based with a large library of available indicators, habitats and ecosystem components. It allows different rules to be used for aggregating indicators, is fully customizable and will determine uncertainty values based on data inputs. The environmental status of a spatial assessment unit is obtained by choosing the marine region, entering the assessment values for the indicators chosen (along with an uncertainty measure and the classification scale) allowing the software to calculate and show the resulting status assessment. The algorithms and intermediate calculations are based on weighted average normalized indicators within specific groups. The NEAT weighting procedure avoids the dominance of certain indicators or habitats or spatial units. Thus, no bias is introduced into the assessment by the choice of the indicators. The tool is being trialed with many different user groups and national authorities. It is freely available for a number of different platforms at http://www.devotes-project.eu/neat/.

CONCLUDING REMARKS

In defining and describing the DPSIR Conceptual Framework, we show how it facilitates management and assessment issues and, through the detailed worked examples, show its particular use with respect to the MSFD. By showing the predominant use of the DPSIR framework and its derivatives as a generic approach to risk assessment and risk management, we emphasize the practical limits of conceptual models and diagrams. Whilst they are of value in an abstract or generic application, the underlying complexity of marine systems means that specific applications cannot be easily shown diagrammatically. Hence, following simple Pressure-Impact linkages, the most straightforward option for assessing specific examples of this conceptual model is to record relationships between successive stages by means of matrices. Subsequently, matrices and linkages can be compiled within a database and interrogated and analyzed by means of interactive data filters. Such an approach facilitates the extraction of information for specific stages of the overall process, which can then be used as the input to other techniques, such as Bow-tie analysis.

In emphasizing the complexity of the marine system, here we show that although creating a system which covers all eventualities (all Activities, Pressures, State changes and Impacts on human welfare and the links between these) is a laudable aim, it is more profitable to focus on a problem-based approach. Hence for any specific area (e.g., a Regional Sea, eco-region, or sub-ecoregion) to determine the ranked priority Pressures based on the number of Activities. Each of these can then be addressed through the proposed DPSIR-Bow-tie linked approach in which we can address the main risks and hazards creating Pressures, and thus the Main Event of concern (Smyth and Elliott, 2014). The challenge for marine management, as shown here, is to apply a linked DPSIR approach for the area being managed. By focusing on the risk assessment approach, i.e., the Pressures as mechanisms causing the State changes and Impacts on Human Welfare (and so ultimately impacting on Ecosystem Services and Societal Benefits, *sensu* Atkins et al. (2011)), then by definition management measures for prevention and mitigation/compensation can be implemented; hence the latter being the Responses under DPSIR and the means by which the Responses address the Drivers and Pressures (and State changes) becomes the risk management framework (see Elliott, 2014).

A further challenge, again given the complexity of the marine system, its uses and users, is its ability to respond to exogenic unmanaged Pressures as well as the endogenic managed Pressures where current assessments rarely consider climate changes, although its effects may be implicit in the measurement of indicators. Hence management not only has to provide the Responses to the causes and consequences of change due to system internal Pressures but also the Responses to the consequences of external Pressures. Because of this, the application of the proposed scheme to cumulative and in-combination Pressures, as discussed here, is also an imminent challenge.

AUTHOR CONTRIBUTIONS

CS led the work on this manuscript. CS, KP, SB, KM, ME comprised the core writing team with inputs from JP, OS, SL, NB, AB. All authors were involved in reviewing and editing the manuscript.

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frontiers in Marine Science

REVIEW
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A Global Review of Cumulative Pressure and Impact Assessments in Marine Environments

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Ever more extensive use of marine space by human activities and greater demands for marine natural resources has led to increases in both duration and spatial extent of pressures on the marine environment. In parallel, the global crisis of decreasing biodiversity and loss of habitats has revitalized scientific research on human impacts and lead to methodological development of cumulative pressure and impact assessments (CPIA). In Europe alone, almost 20 CPIAs have been published in the past 10 years and some more in other sea regions of the world. In this review, we have analyzed 40 recent marine CPIAs and focused on their methodological approaches. We were especially interested in uncovering methodological similarities, identifying best practices and analysing whether the CPIAs have addressed the recent criticism. The review results showed surprisingly similar methodological approaches in half of the studies, raising hopes for finding coherence in international assessment efforts. Although the CPIA methods showed relatively few innovative approaches for addressing the major caveats of previous CPIAs, the most recent studies indicate that improved approaches may be soon found.

Keywords: human activities, pressures, multiple stressors, cumulative effects, impacts, ecosystem-based management

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INTRODUCTION

Globally, the marine environment is at risk from multiple human activities such as overfishing, chemical contamination by hazardous substances, inputs of nutrients, physical modification, etc., in addition to climate change, leading to impaired environmental conditions (Lotze et al., 2006). Increasing human pressures leads to decreasing biodiversity and loss of habitats. A greater awareness of these problems has revitalized the scientific research on human impacts and led to an increasing number of laws, strategies and commitments to reduce human impacts on the ecosystem. The challenge for the scientific community lies in showing evidence of the causalities between human activities, the pressure they cause and the associated impacts on species and habitats, including humans and the human society. In the marine environment, the global assessment of human impacts by Halpern et al. (2008) fostered a wave of impact assessments in the world's seas (e.g., Selkoe et al., 2009; Ban et al., 2010; Korpinen et al., 2012). Although many of these assessments followed the same methodology as in the global assessment, new approaches were also found (e.g., Andersen and Stock, 2013; Knights et al., 2013), old approaches were re-assessed (e.g., van der Wal and Tamis, 2014) and spatial accuracy of the assessments increased (e.g., Ban et al., 2010). In this review, we have assessed 40 recent marine assessments of cumulative pressures and impacts and focused on the methodological approaches. We were especially interested in discovering methodological similarities, identifying good practices and proposing areas in need of more robust scientific input.

So-called cumulative pressure and impact assessments (CPIA) aim to cover additive, synergistic and antagonistic effects of multiple pressures on selected features of the ecosystem. In their fullest form, they attempt to cover all existing anthropogenic pressures and estimate their impacts on a wide spectrum of ecosystem components (e.g., Korpinen et al., 2012). More focused CPIAs assess specific species (Certain et al., 2015; Marcotte et al., 2015), communities (Giakoumi et al., 2015) or are limited to specific human activities (Benn et al., 2010) or pressures (Coll et al., 2016). The selection of ecosystem components in CPIAs is an important step, at least in case of selecting characterizing species to represent ecosystems, food webs or habitats, and hence, this review will also analyse the assessment methods in this respect.

The complexity of CPIAs has led to simplistic assumptions in the methods. Halpern and Fujita (2013) listed many of those and discussed the consequences of the assumptions for the overall assessment conclusions. For instance, many methods assume additivity of impacts, while meta-analytical studies indicate strong roles by synergistic and antagonistic effects (Crain et al., 2008). Similarly, the CPIAs analyzed typically assume that the impacts increase linearly with increasing pressures, while non-linear responses seem to be more common in nature (Hunsicker et al., 2016). Despite these assumptions the CPIAs have provided robust outcomes which seem to correlate with the state-of-the-environment assessments (Andersen et al., 2015) and have potential to inform management decisions.

CPIAs are primarily meant to inform decision-makers and guide management decisions. Therefore, the impacts should be traceable all the way to the human activities at sea, on the coast or in in the upstream catchments. Established links between human activities, pressures and ecosystem components are essential for effective and reliable CPIAs. These links are formed on the basis of causality (i.e., which human activities cause which pressures and which ecosystem components do they affect?), spatial overlap, or exposure (i.e., where are the activities, pressures, and ecosystem components located? Is uncertainty considered? How do the pressures decay from their source?) and sensitivity (i.e., how sensitive is a given ecosystem component to a specific pressure?). So far, only a few attempts to link these in a generalized and systematic way have been published (Knights et al., 2013, 2015) but some linkage frameworks have been in use by regional sea conventions for years (e.g., the North-East Atlantic, the Baltic Sea). Solid basis and transparent communication of these links is crucial for taking the message from the scientific community to the decision-making level. The progress in spatial data tools and online map services will certainly help in that task. Nonetheless, this review critically evaluated the activity-pressure-impact links of the CPIAs.

The cornerstone of CPIAs is the estimation of the potential impact of a specific pressure on a specific ecosystem component. This has been estimated numerically on the basis of spatial damage or loss of individuals (e.g., Giakoumi et al., 2015; Coll et al., 2016) or categorically on the basis of literature reviews and expert panels (e.g., Halpern et al., 2007; Eno et al., 2013). The potential concerns with such a variety of approaches are, firstly, if the different estimate variables are comparable, and secondly, whether the validation (referred to by some authors as "ground-truthing") of the CPIAs to realistic "effect scales" is reliable. To our knowledge this is the first scientific review of the CPIAs and as such its general aim is to lay down an overview of the existing methods and practices.

MATERIALS AND METHODS

Scope of the Review

This review has the general aim to provide an overview of the methods and practices that are used to produce CPIAs in marine environments. It will not evaluate input data or assessment practices outside the methods, even though these may, nonetheless, have important functions in communication, transparency, and confidence of the assessments.

This review has five specific objectives: (1) To compare and find similarities in the structures of the CPIA methods; (2) to evaluate the selection of ecosystem components included in CPIAs; (3) to evaluate the links between human activities, pressures and associated impacts in the CPIAs; (4) to compare the methods in estimating potential impacts; and (5) to find good practices in validating the CPIAs. Each of these objectives is met by defining a number of research questions to be answered for each of the reviewed studies. The research questions are given in **Table 1**.

Selection of Studies

We reviewed CPIA studies which have been published after 2000 and included integration of at least two different pressures. We accepted studies which assess cumulative pressures or cumulative impacts but did not include concept papers unless they piloted a case study or gave an operational method formulation. We performed this search globally by the Google Scholar engine with key words "cumulative effect [/impact] on marine environment [/ecosystem]," "marine cumulative impact assessment," and "Halpern impact assessment of marine pressures." The search was limited to the period 2000–2016 and the results were asked in the order of relevance. The search gave thousands of matches, but we analyzed only 750 first hits and applied the above-mentioned exclusion/inclusion criteria. We also included studies which were cited in the found CPIAs and matched with our search criteria. In total, 35 peer-reviewed CPIA studies were found. However, we also noticed that many CPIA studies have been published as project reports or in institutions' report series due to the nature of this assessment field and those assessments included interesting methodological development. Therefore, we included five additional studies. Hence, our review included altogether 40 studies. Global distribution of the studies is given in **Figure 1** and full references to the studies are given in the Supplementary Material (Appendix A).

TABLE 1 | Specific research questions in the review.

1. Compare and find similarities in the structures of the CPIA methods
1.1 Does the method assess impacts or pressures?
1.2 What integration method the CPIAs have (separate, additive, synergistic, antagonistic)?
1.3 What is the form of pressure—impact relation (categorical, linear, non-linear)?
2. Evaluate the selection of ecosystem components into CPIAs
2.1 Does the CPIA include impacts on species?
2.2 Does the CPIA include impacts on benthic habitats?
2.3 Does the CPIA include impacts on pelagic habitats?
3. Evaluate the links between human activities, pressures and associated impacts in the CPIAs
3.1 Does the CPIA assess human activities?
3.2 Does the CPIA provide activity—pressure links?
3.3 Does the CPIA aggregate pressures from >1 human activities?
3.4 Is the CPIA built on an entire linkage framework of activities, pressures and ecosystem components?
3.5 Does the CPIA benchmark pressure levels for impact estimates?
3.6 Does the CPIA provide a maximum value for pressures?
4. Compare the methods in estimating potential impacts
4.1 Are impact estimated based on expert judgment?
4.2 Are impact estimated based on literature reviews?
4.3 Are impact estimated categorical or continuously numerical?
4.4 Are impact estimates derived from a model?
5. Find good practices in validating the CPIA
5.1 Have the impact results been validated, i.e., anchored into specific state of the environment?
5.2 What is the validation method?

The questions are categorized under the five objectives of the review.

Evaluation Criteria

Each of the studies were analyzed to find answers to the five specific objectives and research questions (**Table 1**). The five objectives were evaluated generally following the descriptions of the reviewed study methods but also a more specific analysis of the methods was made in order to see tabular summary information of the recent CPIAs and compare them against major assumptions of the CPIAs as listed by Halpern and Fujita (2013). In case of the cumulative pressure studies, we evaluated only the general structure (objective 1) and links between activities and pressures (objective 3), as the other objectives require an impact assessment. Full results of the analyses are annexed as Supplementary Material (Appendix A).

Defining the Terms

The scientific literature provides a wide range of terms for CPIAs. An extensive discussion on this is given by Judd et al. (2015), who also provide definitions for the whole pathway from sources (e.g., human activities) to pressures, effects, receptors (e.g., ecosystem components), and impacts. In this study, we use the term "human activity" instead of "source" and define "pressure" (following Judd et al., 2015) as "an event or agent (biological, chemical, or physical) exerted by the source to elicit an effect." Although an effect and an impact can be defined as different steps on the pathway, we have chosen to use the term "impact" in this review. This is a pragmatic solution as our reviewed literature uses both these terms in justifiable way (*sensu* Judd et al., 2015).

RESULTS

Similarities in the Structures of the CPIA Methods

Of the 40 studies reviewed, 33 had assessments of cumulative impacts and seven assessed cumulative pressures. Most of the assessments ($n = 35$, 88%) assumed cumulative pressures or impacts as additive and five assessments included synergistic or antagonistic effects (**Figure 2**). The synergistic and antagonistic effects were mainly assessed in those CPIAs which used ecosystem models, but in one study synergistic effects were inserted into an additive model by defining pressures enhancing the effects of other pressures (Certain et al., 2015). Most of the methods (93%) also assumed linear relationships between activities, pressures and impacts (**Figure 2**). In one assessment the relationship was not clear and in two assessments the relationship was categorical. With the exception of four studies (Aubry and Elliott, 2006; Foden et al., 2011; Giakoumi et al., 2015; Knights et al., 2015), all the others made the assessments with varying spatial resolution (often by 0.2–2.5 km grid cells).

The CPIAs showed relatively similar structures. More specifically, 50% of the studies claimed that they follow the same method as in Halpern et al. (2008) or had a similar method (without directly referring to the Halpern study) (see Appendix A in Supplementary Material). These assessments consisted mainly of three components: (1) intensity of pressures (>1 layers), (2) occurrence of ecosystem components (>1 layers, only if impacts were assessed), and (3) some types of weighting factors to express impacts or to weight pressures. In those studies, where impacts were assessed, a weighting factor was produced for each specific pressure–ecosystem component combination, whereas in the pressure assessments the weighting factors were produced to balance threats between the pressures. The impact weighting factors were sometimes called "vulnerabilities" or "sensitivities" of the ecosystem components to pressures.

In addition, there were a few other methods which relied on similar additive-type models and will likely produce comparable assessment results (e.g., Zacharias and Gregr, 2005; Stelzenmuller et al., 2010; van der Wal and Tamis, 2014). Thus, there seems to be a mainstream approach in the CPIAs which is used worldwide (**Figure 1**), but where small adaptations have been applied in treating of input data and ecosystem sensitivity and in integrating these into the score of cumulative pressures or impacts.

Selection of Species and Habitat Data into the CPIAs

Cumulative impacts were assessed for benthic habitats in 76% of the impact assessments, but also species (41%) and pelagic habitats (38%) were included in the studies (**Figure 3**). Species, benthic habitats and pelagic habitats together were included in only 12% of the studies. Only two studies assessed an entire community, including all the major components to the model (sea grass ecosystem: Giakoumi et al., 2015; 3 exploited fish

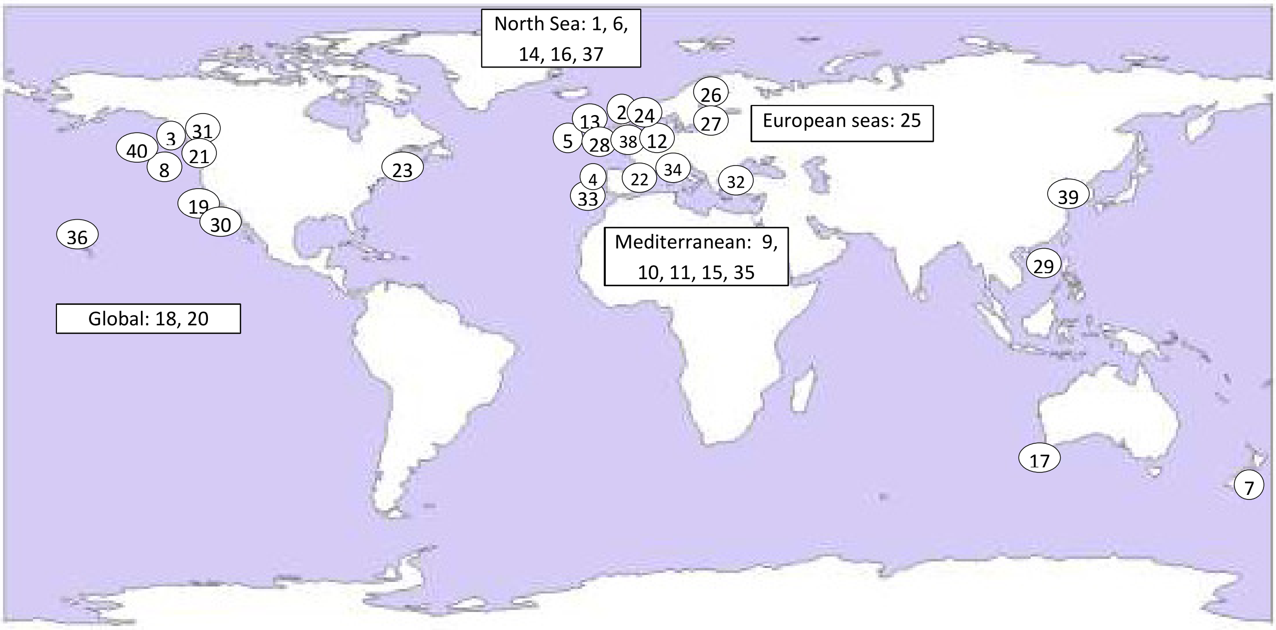

FIGURE 1 | Map with studies included in this review. Global studies and sea areas with several studies are shown in separate text boxes. Key: 1, Eastern North Sea (Andersen and Stock, 2013); 2, U.K. (Aubry and Elliott, 2006); 3, Canada's Pacific (Ban et al., 2010); 4, Portugal (Batista et al., 2014); 5, NE Atlantic (Benn et al., 2010); 6, North Sea (Certain et al., 2015); 7, New Zealand (Clark et al., 2016); 8, Canada's Pacific (Clarke Murray et al., 2015); 9, Mediterranean Sea (Claudet and Fraschetti, 2010); 10, Mediterranean Sea (Coll et al., 2012); 11, Mediterranean Sea (Coll et al., 2016); 12, Netherlands (de Vries et al., 2011); 13, UK (Eastwood et al., 2007); 14, UK (Foden et al., 2011); 15, Mediterranean (Giakoumi et al., 2015); 16, North Sea (Goodsir et al., 2015); 17, SE Australia (Griffith et al., 2012); 18, Global (Halpern et al., 2008); 19, California Current (Halpern et al., 2009); 20, Global (Halpern et al., 2015); 21, Washington US (Hayes and Landis, 2004); 22, French Mediterranean (Holon et al., 2015); 23, Massachusetts (Kappel et al., 2012); 24, Scotland (Kelly et al., 2014); 25, European seas (Knights et al., 2015); 26, Baltic Sea (Korpinen et al., 2012); 27, Baltic Sea (Korpinen et al., 2013); 28, Netherland (Lindeboom, 2005); 29, Hong Kong (Marcotte et al., 2015); 30, California Current (Maxwell et al., 2013); 31, Puget Sound, Canada's Pacific (McManus et al., 2014); 32, Mediterranean Sea and Black Sea (Micheli et al., 2013); 33, Spain (Moreno et al., 2012); 34, Liguarian Sea (Parravicini et al., 2011); 35, Mediterranean Sea (Rodríguez-Rodríguez et al., 2015); 36, Hawaii (Selkoe et al., 2009); 37, North Sea (Stelzenmuller et al., 2010); 38, Noth Sea (van der Wal and Tamis, 2014); 39, Jiaozhou Bay, North Yellow Sea (Wu et al., 2016); 40, (Zacharias and Gregr, 2005). Map from Natural Earth (free vector and raster map data @ naturalearthdata.com).

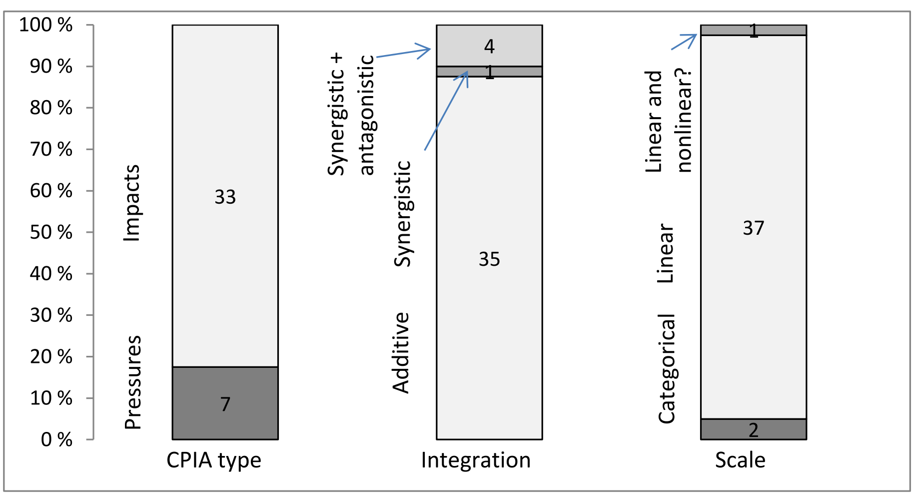

FIGURE 2 | Summary information of the 40 cumulative pressure and impact assessments (CPIA) included in the review. The CPIA type is divided into pressure and impact assessments. The integration was additive, synergistic or synergistic, and antagonistic. The scale of the pressure-impact relationship is divided into categorical, linear and linear and non-linear (with some uncertainty of this indicated by ?-mark).

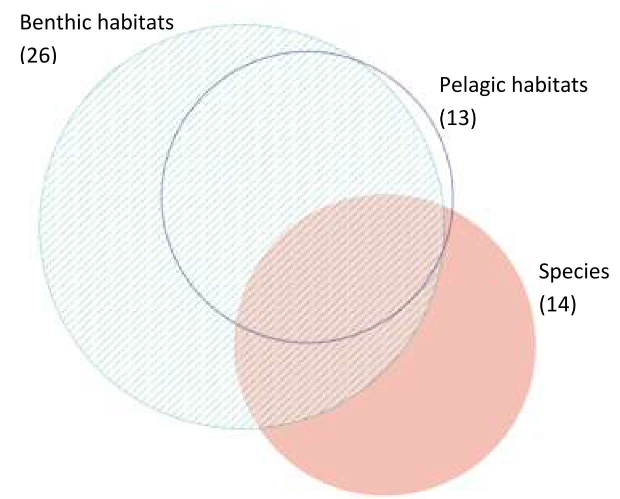

FIGURE 3 | Venn diagram for the inclusion of ecosystem components (species/species groups, benthic habitats, and pelagic habitats) in the reviewed CPIAs. Note that the number of studies in the figure is >40, because a CPIA can assess all the categories. Source of the Venn diagram: EulerAPE (http://www.eulerdiagrams.org/eulerAPE/).

species: Coll et al., 2016). Obviously all of the CPIAs had a limited number of ecosystem components in the assessments, but 21% of them had focused only on a species group (e.g., Zacharias and Gregr, 2005; Coll et al., 2012) or a single species only (Marcotte et al., 2015). However, many of the studies claimed to be demonstration studies and, hence, the selection of ecosystem components was made on practical grounds. Only in one study, a specific justification was given on the grounds of cultural, biological and legal arguments (Hayes and Landis, 2004). Nevertheless, there seemed to be a common lack of precise justification in the reviewed CPIAs, why some species or habitats were selected and others not.

Have the CPIAs Defined Linkages between Activities, Pressures and Impacts?

Ten studies (25%) had defined all the linkages between human activities, pressures and impacts and made a framework to support the CPIA. All of the 10 CPIAs were assessments and not demonstration studies (see Appendix A in Supplementary Material). Additionally, nine more studies had covered all the human activities or all the pressures in the area but not linked them in a systematic way. However, in many cases, it was not possible to estimate whether the systematic framework was made outside the study and used in a more limited way. The review showed that the actual CPIA have taken seriously the linkages between activities, pressures and ecosystem components, often consulting local experts or making extensive literature surveys (e.g., Selkoe et al., 2009; McManus et al., 2014).

In summary of the review results, human activities were included in 31 studies (78%), 26 studies (65%) linked pressures to the human activities and 30 studies (75%) had defined the human pressures into general pressure categories, for instance according to the EU Marine Strategy Framework Directive (MSFD).

Only one study had considered the maximum potential value of pressures (Clark et al., 2016). This is a necessary step in the CPIA procedure if pressures are quantified. Hence, almost all of the reviewed studies assumed that the maximum pressure value in the assessment area is the maximal intensity of that pressure. Moreover, while the majority of studies had normalized the pressure intensities (e.g., 0–1), none of the studies had benchmarked the pressures in order to estimate the impacts in a comparable way (i.e., defined the level of pressure where the impacts occur; see Halpern and Fujita, 2013). One of the studies asked experts to estimate impacts on a "typical level of pressures" (Andersen and Stock, 2013). The lack of definite benchmarks is especially problematic in case of non-linear relation of pressures and impacts. If the relation is non-linear, for instance logarithmic, a relatively low level of pressure can cause high impacts and the magnitude of impact does not increase much at higher pressure levels. However, most of the reviewed CPIAs assumed a linear increase of impacts as a pressure increases. This simplifies the impact formula, where each pressure can be given a single sensitivity score (for each ecosystem component combination).

Estimation of Impacts

We analyzed whether the CPIA studies estimated impacts from anthropogenic pressures by expert judgment or based on scientific literature. Of the 35 studies giving some kind of a weight factor (for impacts or pressures), 23 CPIAs (66%) relied on expert judgment, and 14 (40%) on literature (**Figure 4**). In two studies, the experts were informed by a review of scientific literature (See Appendix A in Supplementary Material).

Impact estimates were most often (69%) categorical expressions of the sensitivity of the ecosystem components to the pressures or severity of the pressures on ecosystem components (**Figure 4**). Continuous impact scales were used in 31% of the studies and in these CPIAs the impacts were often estimated either from a few known parameters, such as mortality (e.g., de Vries et al., 2011), biomass change (Coll et al., 2016), or loss of habitat area (e.g., van der Wal and Tamis, 2014). In these studies, the scope of the CPIA was more limited, focusing on a few ecosystem components (a single species or a species group), of which the impact parameter (e.g., mortality) could be estimated. The more diverse ecosystem components there were in the CPIA studies, e.g., both species and habitats, the more the studies relied on categorical or semi-quantitative impact/sensitivity categories.

Five of the 33 studies (15%), which assessed cumulative impacts, used meta-analyses or an ecosystem model to estimate impacts. The ecosystem models included, for instance, fishing effects on commercially exploited fish species (Coll et al., 2016) and main threats to the seagrass food web (Giakoumi et al., 2015). In one study, pressures were linked to biological quality indicators and the relationship was modeled (Parravicini et al., 2011). This model was used to predict impacts when the pressures were changed.

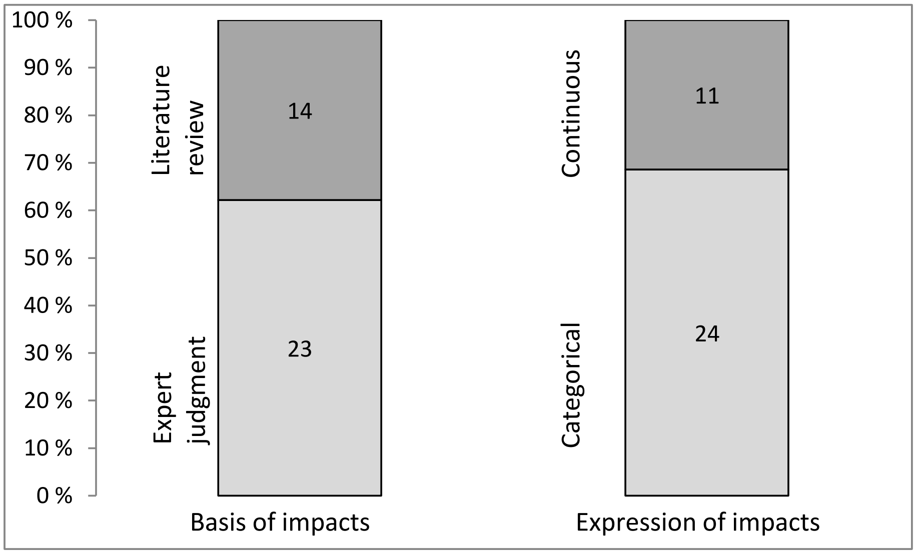

FIGURE 4 | Differences in estimating and expressing impacts of anthropogenic pressures. The impact estimates are based on expert judgment or literature (including models where the interactions are literature-based). The impacts are expressed on categorical scales and on continuous scales. Note that the numbers also include those studies where "impacts" are not specific to ecosystem components but used to weight pressures. Two of the studies used both literature and expert judgment as the basis.

Validation of the Impacts

Only 8 of the 40 studies (20%) had validated the results, i.e., compared the cumulative impact (or pressure) scores with observed environmental status and then re-categorized the impact gradient into a realistic scale (Appendix A in Supplementary Material). However, three of the eight validated CPIAs used a scale obtained from another study and made no reanalysis in their own study. Thus, in reality, only five studies had really validated their impact scores with environmental status assessments. In addition, two more studies indicated how the validation should be made but did not apply it (Zacharias and Gregr, 2005; Claudet and Fraschetti, 2010).

The best description of validation was given by Clark et al. (2016) who compared the cumulative impact scores (on benthic habitats) with benthic fauna data. They found significant relationships between the benthic community composition based on Bray-Curtis similarities and the cumulative impacts by using non-parametric regression (DISTLM). This was also used to test the relation of individual standardized pressures to macro fauna data, without including the habitat sensitivity information to the pressure data. Clark et al. (2016) argue that validation may result in relatively weak relationships if the range of stressor levels is small, which is often the case in local studies. A large-scale validation was applied by Andersen et al. (2015) on a Baltic Sea-wide scale, where cumulative impact scores for sub-basins were compared with integrated state of marine biodiversity. In that scale, the relationship was significant, but due to the small number of sub-basins ($N = 9$), it was not possible to make conclusions about thresholds or tipping points.

DISCUSSION

Identification of marine areas that are sensitive and vulnerable to human activities is not a novelty; environmental sensitivity indices were launched already in the 1970s (Gundlach and Hayes, 1978). Cumulative assessments of multiple pressures and their impacts were carried out already in 1990s (e.g., Wiegers et al., 1998). Methodological development did not, however, receive wide attention until the 2000s when series of CPIAs were produced after the global impact assessment (Halpern et al., 2008). As shown in this review of 36 CPIAs in 2000s, more than half of them were based on the method by Halpern et al. (2008). However, similar research threads had already been started elsewhere (e.g., Lindeboom, 2005; de Vries et al., 2011; van der Wal and Tamis, 2014; Certain et al., 2015) and in comparison to these earlier methods, it is interesting to note that the method presented in Halpern et al. (2008) has allowed wider assessments in terms of human activities, pressures and ecosystem components than the other methods which tend to produce more focused (and sometimes more detailed) assessments in terms of activities, pressures and ecosystem components. Also various ecosystem models have this same limitation.

The review showed that the CPIAs have, in general, three essential components: spatial data on intensity of pressures, spatial data on occurrence of ecosystem components, and factors estimating impacts. In all of the three components, many of the reviewed CPIAs used simplified assumptions (see Halpern and Fujita, 2013) and had small differences in the approaches. Nonetheless, the majority of the studies, at least the ones based on additive integration and estimates of habitat sensitivity, can be expected to produce relatively comparable results and one can see potential improvements to the general method in the most recent studies. Although the 40 reviewed CPIAs were published between 2004 and 2016, 30% of them were from the 2 most recent years and these contained novel approaches more often than the earlier CPIAs. Such approaches were, for instance, the use of fuzzy logic for impact occurrence (Marcotte et al., 2015), building on a fixed linkage framework (Goodsir et al., 2015), separating habitat recovery to a specific assessment (Knights et al., 2015), using food web models (Coll et al., 2016) or other statistical methods (Wu et al., 2016) and describing good practices in validation and pressure quantification (Clark et al., 2016).

Treatment of Spatial Input Data

In the pressure data sets, the main assumptions relate to the spatial extent of pressures from their sources, quantification of the pressures (often on the basis of underlying human activities) and the normalization of the pressures. Spatial extent of pressures has often been treated as a linear decaying model from the source, whereas e.g., Andersen and Stock (2013) produced five alternative models which were used for different types of pressures. The quantification of pressures on the basis of human activities is an assumption which is difficult to replace by real pressure data. No monitoring programme can be expected to measure, e.g., resuspension from bottom-trawling and, hence, fishing activity data is used to estimate the pressure. The pressures are then normalized to a dimensionless scale in order to make them comparable with other pressures, measured in other units. The most frequently used approach was to scale the pressure values linearly such that the highest value is equal to 1.0. Obviously, the main problem with this method is the assumption that the data set contains the maximum value of that pressure. In reality, the pressures in the assessment period may be much lower than the long-term maximum if management measures have been implemented. Among the studies in this review, Clark et al. (2016) was the only CPIA setting a theoretical maximum value for each of the pressure data sets. In addition, Halpern et al. (2015) normalized the pressures according to the highest value of two data sets to allow temporal comparison of two assessment periods.

Occurrence of ecosystem components—species and habitats—in the assessment units determines whether an impact can take place in that area. The occurrence of the habitats was in all the cases reported as presence/absence, whereas for species occurrence probabilities were also applied (Andersen and Stock, 2013). Even though no CPIA used a probability scale for habitat presence, this could be applied if the habitat presence is uncertain due to the low confidence in the input data. Only a few of the reviewed CPIAs (9%) targeted the entire marine ecosystem, i.e., species, benthic, and pelagic habitats. The majority of the studies (55%) focused solely on benthic and pelagic habitats and 21% included species only. Because of the additive approach in most of the CPIAs, a major difference is also the choice to use only benthic habitat layers over the entire assessment area with only one habitat type in a grid cell (e.g., Korpinen et al., 2013) or, alternatively, to use several overlapping layers of ecosystem components and several ecosystem components per grid cell (e.g., Halpern et al., 2008). In the former, the resulting cumulative impacts are relatively simple to interpret, because all the impact scores indicate the amount of pressures, whereas in the latter case one needs to consider also the diversity of ecosystem components in an area when interpreting the cumulative impacts. Both of the approaches are conceptually correct, but they tell slightly different stories from the anthropogenic pressures.

How Vulnerability Is Assessed?

There are basically two types of differences in integrating impacts from multiple pressures: using similar endpoints (same variables) from all the pressures or integrating categorized impacts of different types of variables. In this review, these two basic categories were found and further divided to more detailed sub-types: (1a) categorical expressions of potential impacts on ecosystem components, where the impacts have been usually defined by 3–5 criteria (e.g., functional impact, resistance, recoverability and frequency; e.g., Halpern et al., 2007); (1b) categorical expressions of habitat sensitivity, which has been defined by resistance and resilience (e.g., Stelzenmuller et al., 2010, see also Eno et al., 2013); (2a) numeric estimate of impact by a measurable variable (e.g., proportion of disturbed sea floor; van der Wal and Tamis, 2014, or change in biomass in Coll et al., 2016); and (2b) effect sizes of impacts in a meta-analysis (e.g., Claudet and Fraschetti, 2010). The two former methods are comparable, both considering categorical estimates of sensitivity of the ecosystem component, while the two latter ones use data-based approaches. These latter approaches share the limitation that common parameters are difficult to find for multiple pressures. So far, the quantitative, data-based CPIAs have not been applied to more than a few pressures or ecosystem components, which has limited their usefulness for getting a wider view of human impacts on marine environment.

There has been considerable progress in recent years in developing sensitivity estimates for species and benthic habitats. Zacharias and Gregr (2005) defined the terms sensitivity and vulnerability in an explicit and quantifiable manner with the aim to produce a tool that can predict and quantify vulnerable marine areas (VMA). Using the same or similar definitions, Tyler-Walters and Jackson (1999), Tillin et al. (2010), Eno et al. (2013), and La Rivière et al. (2016) have defined parameters for sensitivity estimates and procedures how these can be assigned to broader habitat types, which are usually the only available mapped marine habitats. Also the meta-analytical approach has been used by Claudet and Fraschetti (2010) to produce data-driven impact estimates for the Mediterranean Sea. Despite the progress, these were used very little, if at all, in the reviewed CPIAs.

Needs for Further Progress in CPIA Methodology

The review showed that none of the CPIAs had benchmarked the pressures (i.e., a quantitative definition of a certain level of pressure, for which the impact or sensitivity is estimated). This is especially problematic for CPIAs which assessed very different types of activities causing same types of pressures. For example, siltation of seabed is caused by laying cables on sea floor, bottom-trawling, dredging and disposal of dredged material (to name a few activities), but the amount of sedimentation varies between the activities, i.e., a low pressure for each activity, if measured by different parameters, may mean different amounts of sediment and, hence, different impacts. This difference in activities was normally addressed in the reviewed CPIAs by giving different sensitivity scores for the pressures from different activities. This is an adequate "fix" if the impacts from pressures increase linearly. However, in non-linear cases, this assumption is no longer valid. This challenge was addressed by Tillin et al. (2010) who proposed to divide pressures to 2–3 sub-pressures based on their magnitude and define benchmarks for these pressures in order to give sharper and more comparable estimates of habitat sensitivity. For example, sea-floor abrasion was sub-divided to "penetration of the seabed surface," "shallow abrasion/penetration of the seabed surface" and "surface abrasion," and benchmarks to these were defined as "$>$25 mm penetration," "$\leq$25 mm penetration," and "surface damage." The approach by Tillin et al. (2010) was taken up by La Rivière et al. (2016) and gives an easily approachable method for CPIAs where habitat sensitivity is defined by expert judgment.

The element of time was not very visible in the reviewed CPIAs. As data sources of human activities and pressures are often imprecise with regard to time of occurrence and duration, the CPIAs assume that pressures are long-lasting and overlap in time. This may well be the case with long-lasting impacts, i.e., with long recovery times, but many of the pressures and impacts are relatively short-lived (e.g., noise, siltation in exposed shores). Such an assumption can be considered as a conservative approach, but some realism could be introduced by specifying impacts seasonally (de Vries et al., 2011) or assessing the potential recovery separately (Knights et al., 2015). A more difficult aspect is the potential accumulation of effects in time (Eastwood et al., 2007). Although difficult to quantify, this was addressed by at least Korpinen et al. (2012) by summing certain pressures over the assessment period when preparing the input data.

An issue in regard to assessing vulnerability which has not been addressed by any of the reviewed studies is the question of historical impacts which have already modified the marine environment. This is especially problematic for the spatial ecosystem data, which only reflects the current situation. In addition, the question of how to assess extinct species or significantly reduced habitat coverage was not addressed by any of the reviewed studies. This specific weakness is something that needs to be solved.

Criticism against the Major Assumptions in CPIAs

Five years after the global map of human impacts (Halpern et al., 2008), a paper was published criticizing the major assumptions in CPIAs (Halpern and Fujita, 2013). The authors listed nine major assumptions in the CPIAs, which are: (1) Stressor layers are of roughly equal importance, (2) Uniform distribution of stressors within a pixel, (3) Habitats either exist or are absent in a pixel, (4) Transforming and normalizing stressors, (5) Linear response of ecosystems to stressors, (6) Consistent ecosystem response, (7) Vulnerability weights sufficiently accurate, (8) Additive model, and (9) Linear response of ecosystems to cumulative impacts. For more detailed description and examples of these assumptions, readers are invited to read the full paper, but here we can briefly analyse how well the studies of this review, especially those published after 2013, have addressed these assumptions.

In this review, we saw that fairly few studies had included the full array of pressures in the assessment. Those that did this had commonly built a linkage framework between activities and pressures and aimed to aggregate pressures from several activities (addressing assumption #1). This is a tedious task if done properly, as described by Tillin et al. (2010). Assumptions #2 and #3 deal with the spatial resolution of input data and these aspects were not included in this review. However, assumptions #4 and #5 relate directly to the core of this review and may cause under- or overestimation of cumulative impacts, as they are related to the estimation of impacts at different pressure magnitudes. According to our review results, none of the studies addressed non-linear responses between pressures and impacts (as far as we were able to interpret the methods). Assumption #6 is about consistent impacts in different areas and within the definitions of the ecosystem components. Although being a critical assumption, none of the reviewed studies really addressed this in their methodology. However, some of the CPIAs were geographically limited and local experts were involved in making the impact estimates (e.g., Selkoe et al., 2009; McManus et al., 2014), which may mitigate the potential error. This does not, however, answer the other side of the assumption that impacts should be consistent within broad habitat definitions (which is definitely a bold assumption). In case of the broad-scale benthic or pelagic habitats, Tillin et al. (2010) and La Rivière et al. (2016) suggest the use of "characterizing species" as targets of the sensitivity estimation, but this has not, to our knowledge, been applied in any published CPIA. Assumption #7 raises the concern that expert-based impact estimates are not coherent or accurate. According to our review, 40% of the studies based these estimates on literature while 66% used expert elicitation. None of the studies claimed any comparison between the two approaches but two studies used both the approaches. Assumptions #8 and #9 have already been discussed in this study, but briefly, 88% of the studies assumed additivity and after 2013 only 3 of the 15 studies included synergistic and/or antagonistic effects. Nevertheless, this can be seen as an improvement in CPIA development, as before 2013 only one of the reviewed studies addressed these effects. The inclusion of non-linear responses to the pressure—impact relationship had not, according to our results, progressed at all.

The current CPIA practices are obviously limited by the scientific knowledge we have today, but there are theoretically unlimited possibilities of impacts on diverse marine environment. To tackle the challenge the methods should focus on keystone species and habitats and build on uncertainty assessment principles and a structured approach to filter and prioritize pressures, impacts and ecosystem components (see Wiegers et al., 1998; Judd et al., 2015). In this review we saw still diverse approaches and non-structured methods but also some positive signs.

CONCLUSIONS AND OUTLOOK

Our review showed that despite rapid method development and several recent publications of CPIA around the world, the assessments still rely on major assumptions which may potentially bias the results (Halpern and Fujita, 2013). Only the most recent studies had started developing methods to address the caveats.

We also showed that the assessment published by Halpern et al. (2008) is gradually developing into a global standard, especially taking some of the recent assessments into consideration. Recalling the concerns raised by Halpern and Fujita (2013), this standard would, however, need new openings such as the inclusion of non-linearity to the models or the use of other types of broad modeling frameworks, e.g., Bayesian Belief Networks, in CPIAs (Uthicke et al., 2016). The direction in the most recent studies indicates that this may indeed be the case in the near future.

In the light of this review, there are currently, in our understanding, no other methods capable to assess the whole range of human impacts than the ones similar to Halpern et al. (2008). Hence, we call not only for a further development of the methodology but also a sharing of tools or codes, such as the open access EcoImpactMapper (Stock, 2016), as this will encourage and support both a short term process focusing on the tools and a long-term process supporting CPIA-based marine ecosystem health assessment as well as evidence-based management.

AUTHOR CONTRIBUTIONS

SK: the main author responsible for the analysis and the results. JA: building the study database, supporting the analysis and text, responsible for visual presentation.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmars.2016.00153

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Better monitoring, better assessment

frontiers in Marine Science

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European Marine Biodiversity Monitoring Networks: Strengths, Weaknesses, Opportunities and Threats

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By 2020, European Union Member States should achieve Good Environmental Status (GES) for 11 environmental quality descriptors for their marine waters to fulfill the Marine Strategy Framework Directive (MSFD). By the end of 2015, in coordination with the Regional Seas Conventions, each EU Member State was required to develop a marine strategy for their waters, together with other countries within the same marine region or sub-region. Coherent monitoring programs, submitted in 2014, form a key component of this strategy, which then aimed to lead to a Program of Measures (submitted in 2015). The European DEVOTES FP7 project has produced and interrogated a catalog of EU marine monitoring related to MSFD descriptors 1 (biological diversity), 2 [non-indigenous species (NIS)], 4 (food webs), and 6 (seafloor integrity). Here we detail the monitoring activity at the regional and sub-regional level for these descriptors, as well as for 11 biodiversity components, 22 habitats and the 37 anthropogenic pressures addressed. The metadata collated for existing European monitoring networks were subject to a SWOT (strengths, weaknesses, opportunities, and threats) analysis. This interrogation has indicated case studies to address the following questions: (a) what are the types of monitoring currently in place? (b) who does what and how? (c) is the monitoring fit-for-purpose for addressing the MSFD requirements? and (d) what are the impediments to better monitoring (e.g., costs, shared responsibilities between countries, overlaps, co-ordination, etc.)? We recommend the future means to overcome the identified impediments and develop more robust monitoring strategies. As such the results are especially relevant to implementing comprehensive and coordinated monitoring networks throughout Europe, for marine policy makers, government agencies and regulatory bodies. It is emphasized that while many of the recommendations given here require better, more extensive and perhaps more costly monitoring, this is required to avoid any legal challenges to the assessments or to bodies and industries accused of causing a deterioration in marine quality. More importantly the monitoring is required to demonstrate the efficacy of management measures employed. Furthermore, given the similarity in marine management approaches in other developed systems, we consider that the recommendations are also of relevance to other regimes worldwide.

Keywords: Marine Strategy Framework Directive (MSFD), biodiversity, Good Environmental Status (GES), regional sea, pressures, SWOT analysis

INTRODUCTION

By 2020, European Union Member States should achieve GES (Good Environmental Status) for their marine waters to comply with the Marine Strategy Framework Directive (MSFD; 2008/56/EC). By the end of 2015, in coordination with the Regional Seas Conventions (RSC), each EU Member State was required to develop a marine strategy for their waters, together with other countries within the same marine region or sub-region. Under the MSFD, reporting on GES should be carried out at a Regional Sea level (although marine sub-regions and subdivisions may be used to take into account the specificities of a particular area), which thus requires broad-scale monitoring with the potential to account for ecosystem level changes in response to both anthropogenic and natural pressures. In order to achieve this, assessment of GES under the MSFD is divided into 11 qualitative descriptors that collectively aim to cover the threats, pressures, and status of the whole marine ecosystem to give a complete picture of environmental status (Borja et al., 2013). Some of those descriptors relate to background conditions, some to pressures and some to impacts on the natural or social systems. Specific requirements of the MSFD include: (i) coordination of monitoring between EU Member States, (ii) that monitoring must be compatible with the EU Water Framework Directive (WFD), and Birds and Habitats Directives, and (iii) that monitoring must incorporate physical, chemical and biological components. It is necessary to consider the fundamental niches (i.e., sea bed, water column, and ice) to which each of these 11 descriptors relate, as well as the biological components (e.g., microbes, fish, see below). The assessment of each aspect of the marine environment requires an indicator (or usually a suite of indicators) to inform on state, and these indicators require data collected through monitoring (Shephard et al., 2015) although existing indicators may potentially leave gaps in current monitoring as new needs arise through the MSFD (Teixeira et al., 2014; Berg et al., 2015). Borja and Elliott (2013) describe monitoring *sensu stricto* as "*the rigorous sampling of a biological, physical and/or chemical component for a well defined purpose, against a well defined end-point*" and state that this may be in relation to the detection of trends away from an accepted starting point, non-compliance with a legal threshold, and/or comparison to standards, baseline or trigger points. However, current environmental management refers to different types of monitoring, all of which serve different purposes, with differing methods and analysis of the results. For example, Elliott (2011) identified 10 types of monitoring, two of which are of specific relevance to the MSFD: (1) Surveillance monitoring which enables the detection of spatial and temporal trends and, where necessary, leads to management action (for example, the detection of climate change trends), and (2) Condition monitoring to determine the present status of an area, and to detect change in condition over time (for example the health of the environment). However, once any deleterious change has been detected then investigative or diagnostic monitoring will be required to determine the cause-effect relationship, again linking to management actions.

The results of these types of monitoring, which each cover a spatial extent and/or a temporal duration and frequency, then requires feedback into management and policy decisions (Gray and Elliott, 2009). It is axiomatic that a system cannot be managed unless it is monitored thus giving data to show the status of the system and the results of the management measures implemented, hence taking all these elements together then requires and produces a monitoring program. Zampoukas et al. (2014) defined a Monitoring Program as "*all substantive arrangements for carrying out monitoring, including general guidance with cross-cutting concepts, monitoring strategies, monitoring guidelines, data reporting and data handling arrangements. Monitoring programs include a number of scheduled and coordinated activities to provide the data needed for the ongoing assessment of environmental status and related environmental targets*." A monitoring program can include one or several monitoring activities, defined as "*the repeated sampling and analysis in time or space of one or more ecosystem components and carried out by an individual agency or institution. Data and marine information are obtained on a routine or specific basis, using sea surveys, remote sensing (i.e., teledetection), ferry boxes, data mining, or any other way*." By expanding the comments of Zampoukas et al. (2014), monitoring programs should have an adequate coverage, in terms of accounting for current pressures and impacts on both natural and social systems but should also be adaptable to address environmental variability associated with emerging issues (see also Scharin et al., 2016). For the purposes of the MSFD, monitoring also needs to be coherent and coordinated, whereby EU Member States within the same region or sub-region follow agreed methods and focus on agreed biotic and abiotic components. This ensures that reporting is comparable across sea areas and can be incorporated into assessing GES at a Regional Sea level (Cavallo et al., 2016).

The nature and scale of marine environmental monitoring within Europe, was assessed within the DEVOTES FP7 project

(DEVelopment Of innovative Tools for understanding marine biodiversity and assessing GES, www.devotes-project.eu). This assessment involved compiling a catalog of marine biodiversity monitoring programs at the regional sea level (focusing on MSFD Descriptors where biodiversity is relevant: D1, biological diversity, D2, non-indigenous species (NIS), D4, food webs, and D6, sea-floor integrity). The catalog highlights:

- the MSFD descriptors and biodiversity components being either directly targeted or indirectly addressed through monitoring under other legislative obligations;
- the specific habitat type targeted;
- particular pressures for which the monitoring was designed;
- the marine regions and sub-regions where particular monitoring activities are taking place;
- the time series and frequency of the data collection, to assess temporal change, and
- collaboration between different countries within and between the RSC.

To meet the requirements of the MSFD in terms of demonstrating GES, a detailed understanding of the above requires answering the following questions: (a) what are the types of monitoring currently in place? (b) who does what and how? (c) is the monitoring fit-for-purpose for addressing the MSFD requirements? and (d) what are the impediments to better monitoring (e.g., costs, shared responsibilities between countries, overlaps, co-ordination, etc.)?

By identifying current monitoring, this exercise aimed to highlight omissions in descriptors, biological components and habitats in particular marine regions or sub-regions and provide a broad overview of the spatial distribution and temporal intensity of monitoring activities. In particular, it aimed to identify programs or combinations of programs that will address the requirements of the MSFD, thus enabling decisions to be made about the cost-effectiveness of future monitoring. This high level assessment of the adequacy of current monitoring, in terms of spatial and temporal scale, in turn will allow the identification of components requiring inclusion in existing monitoring programs or the requirement for the development of entirely new monitoring programs. All of these aspects together constitute what is regarded here as a fit-for-purpose monitoring program.

MATERIALS AND METHODS

Devotes Catalogue of Marine Biodiversity Monitoring Networks

Information was compiled regarding the current status of marine biodiversity monitoring, and in particular of the MSFD descriptors 1, 2, 4, and 6. In order to have an adequate spatial coverage of monitoring networks throughout the European Regional Seas, we first identified monitoring activities within the EU Member States or Regional Seas covered by the DEVOTES partners and then circulated the catalog outside that partnership for completion. Several other countries (EU Member States and third countries) voluntarily and enthusiastically provided information to this catalog. However, those areas with which DEVOTES has a stronger link have a more comprehensive coverage in the catalog (**Figure 1**). The catalog and Patrício et al. (2014) form the basis and common authorship of this manuscript. It is however recognized that monitoring programs in EU Member States are subject to regular amendment/change and as such the catalog requires regular updating to reflect the current status of monitoring activities throughout Europe. The catalog is publicly available at http://www.devotes-project.eu/devotes-release-new-version-catalogue-monitoring-networks/. Despite the slightly incomplete nature, we consider that the catalog provides sufficient coverage to give the main lessons to be learned from this first, broad overview of European monitoring activities. It enables detailed analysis to support the harmonization of monitoring throughout Europe.

The focus of the catalog was on monitoring solely related to biodiversity (i.e., relating to MSFD D1, D2, D4, and D6) and not on determinants for human food provision or quality or physico-chemical aspects (unless the latter are collected as supporting data for biotope characterization and biological parameters).

The catalog is presented in the above site as an EXCEL file containing two main tables:—"*MONITORnetworks catalogue*" and the parameters table "*Param* & *physico-chemical data*." The database is structured into three levels:

(1) *Monitoring program level:* this describes the general features of each monitoring activity, including the program name, the website and the time-series of the monitoring to enable users to find the full details (where available) of monitoring activities, methods, indicators, and parameters associated with a specific program. The geographical scope of each program is indicated through participation at national, EU, Regional Sea or local scale (e.g., for research or a single organization operating in a small area) together with information on the Regional or sub-regional seas to which the program applies.

The MSFD descriptor, the biodiversity component and the specific habitat type targeted by each program were identified to allow an assessment of the extent to which current monitoring practices address the ecological components. The biodiversity components include Microbes, Phytoplankton, Zooplankton, Angiosperms, Macroalgae, Benthic Invertebrates, Fish, Cephalopods, Marine Mammals, Reptiles, and Birds. The choice of biodiversity components was based on official MSFD documents and a related Commission Staff Working Paper (EC, 2012). The habitats (fundamental niches) include Seabed, Water column and Ice habitat. The categories adopted for habitat types followed the EU Commission Decision (EC, 2010) and EU Commission Staff Working Papers (EC, 2011, 2012) where it was agreed that the "*use of these types provides a direct link between habitats assessed under Descriptor 1 and the substratum types to be assessed for Descriptor 6) and the European EUNIS habitat classification scheme*" (EC, 2011, p. 18). In each case, the associated physico-chemical data collected (in the *Param* &

FIGURE 1 | Countries that have information reported in the DEVOTES Catalogue of Monitoring Networks (green) by June 2014 (country borders from Natural Earth database, http://www.naturalearthdata.com).

physico-chemical data table) and details of analytical quality control and quality assurance (AQC/QA, e.g., Gray and Elliott, 2009) were highlighted. Including this information broadly indicates the level of detail, confidence in and quality of a monitoring program, giving information on the nature of the explanatory variables, which may be linked to changes in environmental status. In addition, the information contained in these fields provides the opportunity to link the monitoring activities reported in this catalog to the "Data requirements" fields of the DEVOTES Catalogue of Indicators (Teixeira et al., 2014; available at http://www.devotes-project.eu/devotool/).

The extent to which each program accounts for specific pressures (either directly or indirectly where the biological and physico-chemical parameters indicate environmental change associated with those specific pressures) was identified. Here a pressure was defined as "*the mechanism through which an activity has an actual or potential effect on any part of an ecosystem,*" (Robinson et al., 2008; Scharin et al., 2016). There was a list of 37 pressures, several of which were categorized as local and/or manageable if they were considered to occur as a result of human activities taking place on a localized scale and within the management unit (i.e., a discharge, a specific dredge disposal or aggregate extraction site). The causes and consequences of these pressures can be managed through permits/consents and monitoring. They are referred to as "Endogenic Managed Pressures" where the causes are managed as well as the consequences (Elliott, 2011). In contrast, other pressures were categorized as widespread and/or unmanaged, i.e., those that are beyond the control of direct management that are occurring at regional scales and often outside the management unit. For example, temperature and hydrological changes associated with climate change, or pH change due to volcanic activity (which may be local, but is not manageable). These are referred to as "Exogenic Unmanaged Pressures" where the consequences are managed rather than the causes (Elliott, 2011; Scharin et al., 2016). The MSFD only refers to an incomplete list of endogenic pressures and so the DEVOTES pressures list was produced as a revision from the MSFD and Koss et al. (2011). This adds in the managed and unmanaged pressures, thus allowing climate change to be considered as it has been omitted in MSFD implementation despite the wording of the Directive (Elliott et al., 2015).

(2) *Monitoring networks level* (group of monitoring programs undertaken or used within broader programs, such as International Conventions, Regional Sea, EU Directives and/or national programs): this entry includes fields relating to cooperation between countries. This level aims to determine whether the monitoring programs undertaken are within a monitoring network of institutions and, if so, what is the monitoring network name, and which other countries are involved in that monitoring network.

(3) *Web-platform level*: includes details of data provision such as whether the monitoring program provides data to, or takes data from, any international web platform. This level allows the distinction between data sets which are collated in widely accessible formats (i.e., data portal) and those that are collated and stored by individual agencies (these may or may not be accessible on request).

The rationale behind gathering information at the network and web-platform level was to be able to infer whether and if so how EU Member States are optimizing their monitoring plans and efforts.

Data and Information Analysis

The metadata collated in the catalog were subject to a gap analysis to determine missing aspects and whether the current monitoring is fit-for-purpose both in terms of addressing the MSFD requirements but also wider issues within the marine environment such as providing information for maritime spatial planning, blue growth and industrial marine uses. The monitoring programs undertaken within each Regional Sea (and marine sub-region) were collated and assessed against the descriptors, biodiversity components, habitat types, and pressures to identify any gaps in provision. This led to a SWOT (Strengths, Weaknesses, Opportunities, Threats) analysis to better understand the monitoring networks in Europe, thus allowing us: (1) to explore possibilities for new efforts or solutions to problems specific to the MSFD; (2) to identify opportunities for success in the context of threats to success, clarifying directions and choices, and (3) to make recommendations to overcome the identified impediments and develop more robust monitoring strategies for the future.

Both the gap and the SWOT analyses were performed per marine sub-region (where applicable), marine region and at the Pan-European scale (i.e., considering all the activities reported in the catalog). This comprehensive compilation and interrogation allows us to present the main findings that are illustrated by appropriate case studies. More details regarding Regional Sea specific results are given in Patrício et al. (2014, 2015).

RESULTS AND DISCUSSION

What Are the Types of Monitoring Currently in Place?

A total of 57 Institutes (including a significant number from outside the DEVOTES project) provided information on monitoring activities. The catalog considers the depth and extent of marine monitoring in 16 EU Member States (Bulgaria, Croatia, Cyprus, Denmark, Finland, France, Germany, Greece, Italy, Lithuania, Malta, Portugal, Romania, Slovenia, Spain, and United Kingdom) and 15 non-EU countries (Albania, Algeria, Egypt, Georgia, Israel, Lebanon, Libya, Montenegro, Morocco, Norway, Russia, Syria, Tunisia, Turkey, and Ukraine) that share European Regional Seas boundaries. The catalog contains 865 entries (i.e., monitoring activities) and >298 monitoring programs (some of them with several activities). These activities covered four marine regions (Baltic Sea, Black Sea, Mediterranean Sea, North Eastern Atlantic), 23 sub-regions (as they appear in the MSFD Guidance documents e.g., Bay of Biscay and the Iberian Coast, Greater North Sea, Ionian Sea and the Central Mediterranean Sea, Levantine Sea, etc.), 83 ecological assessment areas (as they appear in national and regional documents e.g., Celtic Sea North, Kattegat and Skagerrak, Northern Adriatic) and also included 37 entries for non-EU waters.

Despite biological monitoring in the Baltic Sea starting in 1979 and being carried out annually in all nine surrounding countries, it was not possible to have an adequate coverage of these monitoring activities in the DEVOTES catalogue. Hence, data reported for the Baltic Sea were deemed insufficient to allow a robust analysis of regional biodiversity monitoring networks. This was mainly due to the low number of partners from the Baltic region in the DEVOTES project, whereas at the same time representatives from the Baltic countries were also involved in another regional pilot project (BALSAM, http://www.helcom.fi/helcom-at-work/projects/balsam) for enhancing the capacity of the Baltic Sea Member States to develop their monitoring programs. The BALSAM project was led by HELCOM, the Regional Sea Convention responsible for coordinating monitoring and assessment of the marine environment in the Baltic Sea. The HELCOM Monitoring and Assessment Strategy (MAS) was endorsed by HELCOM HOD 41/2013 and was adopted by the HELCOM Ministerial Meeting in 2013. A review of monitoring programs resulted in the report and publications (HELCOM, 2013, 2015) and so to complement the scarce regional information obtained from the DEVOTES catalogue, we also used data compiled by HELCOM (2013, 2015). We acknowledge the methodological inconsistency in respect to other European marine regions but we considered that it was more acceptable to use these comprehensive reports on the monitoring programs in the Baltic Sea, rather than excluding it. Given the large degree of coordination by the HELCOM countries, in assessing the monitoring activities we assumed that there would be a maximum number of national monitoring programs performed by all Baltic countries (i.e., nine programs) for any element monitored by all states.

Regarding monitoring types, most monitoring reported in the catalog comes under the term surveillance monitoring, ranging from 88 to 94% in the North Eastern Atlantic (NEA), Mediterranean and Black Sea (**Figure 2**). There is less condition monitoring which ranged from 6 to 10% in these three regional seas.

The date at which monitoring started varies widely throughout the catalog (**Figure 3**) but in general the number of monitoring activities has increased over the last 100 years, with most over the last three decades. Important triggers for monitoring were the Regional Sea Conventions and associated Action Plans. However, there are large differences between Regional Seas, for example, compared to the Baltic Sea and North East Atlantic which had monitoring from the 1970s, there are few monitoring activities in the Mediterranean Sea prior to the 1990s and most Black Sea monitoring programs were initiated in the 2000s.

Throughout the catalog, very different monitoring frequencies are reported, varying from minute to sub-hour, hourly, daily, weekly, twice a month, monthly, bi-monthly, 3–6 times a year, seasonally, 2/3 times a year, twice a year, annual, bi-annual, every 6 years, and up to every 10 years to sporadic, depending on which biodiversity component is the target, the national and international environmental regulations and the budgetary constraints.

Who Does What and How?

The catalog identified 298 monitoring programs that are suitable to address GES of the MSFD descriptors (i.e., directly or indirectly target the biodiversity-related descriptors). In the NEA, 60% of monitoring programs are undertaken to fulfill the objectives of European Directives, the OSPAR Convention and other International Conventions (**Table 1**). Thirty-one percent of these programs address two or more of these legislative drivers and 18% additionally address national monitoring obligations (**Table 1**). Most (83%) of these monitoring programs are undertaken by government agencies and institutions, but 17% are also undertaken by charities, Non-Governmental Organizations (NGOs) and research institutes (e.g., SAHFOS in Plymouth coordinates the Continuous Plankton Recorder scheme, which has been monitoring plankton since the 1920s and produces most of the data required for plankton in the UK; **Table 1**). Most of the programs are surveillance monitoring programs (80%) and generally employ common monitoring protocols, particularly where these programs are undertaken within collaborative monitoring networks [e.g., in the UK the Clean Seas Environment Monitoring Program (CSEMP) previously the National Monitoring Plan (NMP) and the National Marine Monitoring Plan (NMMP)]. In the NEA, 38% of monitoring programs are undertaken as part of research programs (e.g., MESH–Mapping European Sea beds Habitats, MISTRALS and French POPEX research programs) and/or to address national monitoring obligations (**Table 1**). These are undertaken by both government agencies (53%) and NGOs and research institutes (46%) and are all surveillance monitoring programs (**Table 1**).

In the Mediterranean Sea, 55% of the monitoring programs are undertaken because of European legislation [e.g., DCR (Data Collection Framework for the EU Common Fisheries Policy) and WFD; **Table 1**]. Of these, 13% addressed two or more legislative drivers and/or research projects. Most programs (66%) are undertaken by government agencies and institutes (**Table 1**). The remaining programs are undertaken by NGOs and research institutes and address basin wide issues or more local research projects (e.g., JellyWatch—CIESM Monitoring jellyfish blooms along Mediterranean coasts and in the open sea or NETCET—Network for the conservation of Cetaceans and Sea Turtles in the Adriatic) and national monitoring (**Table 1**).

In the Baltic Sea, all of the monitoring programs are undertaken to fulfill the objectives of European Directives, the HELCOM Convention and other International Conventions (**Table 1**). Most programs (93%) address two or more of these legislative drivers in addition to national monitoring obligations

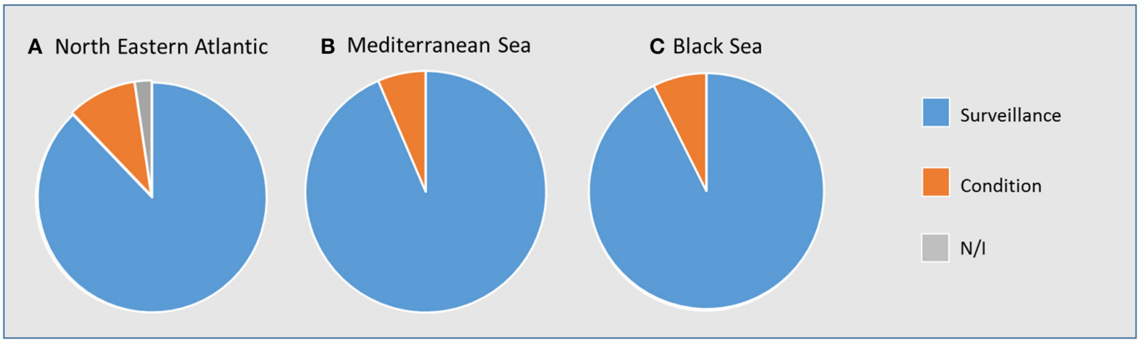

FIGURE 2 | Types of monitoring: condition and surveillance monitoring in the (A). North Eastern Atlantic, **(B)**. Mediterranean Sea and **(C)**. Black Sea. N/I, not identified.

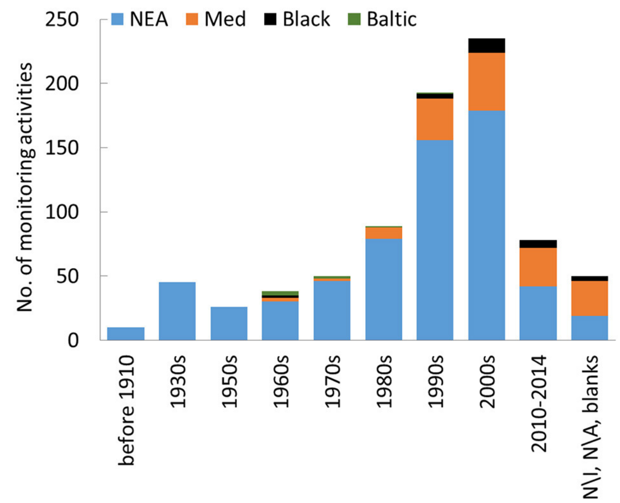

FIGURE 3 | Histogram showing the decades when monitoring activities started in the North Eastern Atlantic (NEA), Mediterranean Sea (Med), Black Sea (Black), and Baltic Sea (Baltic). N/I, not identified, N/A, not applicable.

and, in two cases, research programs. As such, most programs are part of monitoring networks and employ standard monitoring and QA protocols (i.e., HELCOM COMBINE, available at http://www.helcom.fi/action-areas/monitoring-and-assessment/manuals-and-guidelines/combine-manual). These programs are mainly undertaken by government agencies.

In the Black Sea, most monitoring programs (78%) address the objectives of European Directives, the Bucharest Convention and other International Conventions in addition to national monitoring and research programs (e.g., World Ocean–in Russia; **Table 1**). Seventy percent of the monitoring programs are undertaken by governmental agencies and institutes, however 30% of monitoring is carried out by NGOs and research institutes (**Table 1**).

Is the Monitoring Fit-For-Purpose for Addressing the MSFD Requirements?

In the context of the MSFD implementation, as a first step in the preparation of programs of measures, EU Member States across a marine region or sub-region should analyze the characteristics, pressures and impacts in their marine waters (see MSFD Annex III and Commission Decision 2010/477/EU). The second step toward achieving GES should be to establish environmental targets and monitoring programs for ongoing assessment, enabling the state of the marine waters to be evaluated on a regular basis. Hence, it is necessary to question how the monitoring fitness-for-purpose should be assessed. Monitoring has to provide the data to classify a marine area as reaching or failing to reach GES. To do so, the monitoring programs have to accommodate the descriptors, indicative characteristics, pressures, impacts and ideally should be able to provide data for the calculation of the indicators on which GES should be defined. Overall, our analysis showed several areas where current monitoring might not be fit for purpose to address the MSFD requirements.

GES Descriptors

Monitoring programs which address the descriptors D1–biological diversity and D4–food webs are the most numerous in all Regional Seas when taken as a whole, whereas monitoring associated with D2–NIS and D6–seafloor integrity are the least numerous (**Figure 4**). The distribution of monitoring programs that address these descriptors, however, varies both within and between Regional Seas. In the NEA for example, all descriptors are covered by a large number of monitoring programs in the Greater North Sea and Celtic Seas, however monitoring programs in the Bay of Biscay and the Iberian Coast are less numerous and the limited number of monitoring programs in the Macaronesian biogeographic region is of concern. In the Mediterranean, most of the 35 cataloged activities addressing descriptor D4 have been carried out in the Western Mediterranean, whilst only a limited number of monitoring activities currently addresses this descriptor in the Central and Eastern Mediterranean. In the Black Sea, descriptor D4 is the least monitored descriptor and only three monitoring activities cover it. Regarding monitoring of descriptor D2, few monitoring activities have been reported in all Regional Seas apart from the Greater North Sea and Celtic Seas of the NEA.

Some of the above highlighted gaps were expected. For example, monitoring for non-invasive species was not explicitly required by EU law before the MSFD entered into force although some EU Member States have been collecting data on non-invasive species and using them for coastal water quality assessment. The lack of D2 monitoring agrees with Vandekerkhove and Cardoso (2010) that most monitoring programs fail to detect some indicative NIS. Zampoukas et al. (2014) recommended that existing monitoring programs (e.g., for the WFD) should be complemented to explicitly record NIS and to include high priority sampling sites. Descriptor D6 is covered in all Regional Seas and sub-regions, apart from the Maraconesia biogeographic sub-region where D6 monitoring is lacking, which represents a major gap. Until recently, technical difficulties associated with deep sea sampling (Diaz et al., 2004) and a lack of tradition arising from the absence of effective international measures for assessing and protecting those habitats (Davies et al., 2007) explain why these habitats lag behind in established and complete monitoring programs. This explains why regions dominated by open sea and deep-sea ecosystems may have a poor data availability and hence face a greater difficulty in addressing MSFD D6 requirements.

Biodiversity Components

In general, monitoring programs which address high trophic level biodiversity components (such as reptiles, mammals, and birds) are lacking or limited in some Regional Seas (e.g., Black Sea and Mediterranean Sea) compared to the NEA (**Figure 5**). Cephalopod monitoring is limited in all Regional Seas. Monitoring programs addressing fish were not identified as lacking or limited in any Regional Seas although that monitoring is not evenly distributed throughout the sub-categories, with

TABLE 1 | European Directives, Regional Sea Conventions, International Conventions, and National Monitoring projects addressed through monitoring programs in the North Eastern Atlantic, Baltic Sea, Mediterranean Sea, and Black Sea.

European directives	Regional Sea conventions	International conventions, agreements, and organizations	National monitoring	Monitoring parties (Government Agencies/Institutions and Public Bodies)	Monitoring parties (Research Institutes, Industry, Charities and NGOs)
NORTH EASTERN ATLANTIC (NEA)					
Common Fisheries Policy (CFP), Habitats Directive (HD), Birds Directive (BD), Water Framework Directive (WFD), Dangerous Substances Directive, Nitrates Directive, Urban Waste Water Treatment Directive (UWWTD), Shellfish Hygiene Directive, Natura 2000, Marine Strategy Framework Directive (MSFD)	OSPAR Convention (OSPAR), Helsinki Convention (HELCOM)	Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), International Council for the Exploration of the Sea (ICES), International Whaling Commission (IWC), African-Eurasian Waterbirds Agreement (AEWA), Convention on Wetlands of International Importance (RAMSAR Convention), Agreement on the Conservation of Small Cetaceans in the Baltic, North East Atlantic, Irish and North Seas (ASCOBANS) Convention on Biological Diversity (CBD)	**UK:** NI Wildlife Order, UK Wildlife and Countryside Act, UK Biodiversity Action Plan, UK Marine Science Strategy, Scottish Marine Science Strategy, Marine (Scotland) Act, UK Wildlife and Countryside Act, Conservation of Seals Act (GB) **Denmark:** National Monitoring for Aquatic and Terrestrial Environment (MOVANA) **Portugal:** National program of biological sampling (PNAB)	**UK:** Agri-Food and Biosciences Institute (AFBI), Northern Ireland Environment Agency (NIEA), Environment Agency (EA), Center for Environment, Fisheries and Aquaculture Science (Cefas), Natural Resources Wales (NRW), Scottish Natural Heritage (SNH), Marine Scotland Science (MSS), Joint Nature Conservation Committee (JNCC), Scottish Environment Protection Agency (SEPA), Natural England (NE), Plymouth Marine Laboratory (PML), UK National Oceanography Center (NOC), Non-Native Species Secretariat (NNSS) **France:** French Research Institute for Exploitation of the Sea (IFREMER), French National Center for Scientific Research (CNRS), French Agency for Marine Protected Areas **Portugal**: Portuguese National Meteorological Service (IPMA), Portuguese Task Group for the Extension of the Continental Shelf (EMEPC) **Norway:** Norwegian Environment Agency **Denmark:** Danish Ministry of the Environment (MIM) **Spain:** Basque Water Agency (URA), Spanish Institute of Oceanography (IEO)	**UK:** Sea Mammal Research Unit (SMRU), Scottish Association for Marine Science (SAMS), British Trust for Ornithology (BTO), Wildfowl, and Wetlands Trust (WWT), Zoological Society of London (ZSL), University of Liverpool, Sea Watch Foundation, Marine Biological Association (MBA), Hebridean Whale and Dolphin Trust **Portugal:** Portuguese Society for the Study of Birds (SPEA) **France:** CEMEX, Center for study and promotion of algae (CEVA)
BALTIC SEA					
Habitats Directive, Birds Directive, Water Framework Directive (WFD), Marine Strategy Framework Directive (MSFD), Shellfish Hygiene Directive, Nitrates Directive, Common Fisheries Policy (CFP)	Helsinki Convention (HELCOM)	Stockholm Convention on Persistent Organic Pollutants (Stockholm Convention), United Nations Economic Commission for Europe (UNECE), International Council for the Exploration of the Sea (ICES), Convention on Wetlands of International Importance (RAMSAR Convention), International Maritime Organization (IMO), United Nations Environment Program (UNEP)	**Lithuania:** National Monitoring	**Finland:** Finnish Environment Institute **Lithuania:** Environmental Protection Agency **Germany:** Federal maritime and hydrographic Agency (BSH)	

(Continued)

TABLE 1 | Continued

European directives	Regional Sea conventions	International conventions, agreements, and organizations	National monitoring	Monitoring parties (Government Agencies/Institutions and Public Bodies)	Monitoring parties (Research Institutes, Industry, Charities and NGOs)
MEDITERRANEAN SEA					
Water Framework Directive (WFD), Bathing Water Directive (BWD), Common Fisheries Policy (CFP)	Convention for the Protection of the Marine Environment and the Coastal Region of the Mediterranean (Barcelona Convention)	Scientific Cooperation to Support Responsible Fisheries in the Adriatic Sea (FAO-AdriaMed Project), Convention for the United Nations Environment Program Mediterranean Action Plan for the Barcelona Convention (UNEP/MAP), International Waterbird Census (Wetlands international monitoring; IWC)	**France:** French natural reserves monitoring (RNF), Marine stations network plankton survey (RESOMAR) **Turkey:** Integrated Marine Monitoring Program **Greece:** LARCO and Saronikos **Israel:** Israel Oceanographic and Limnological Research (IOLR)	**Spain:** Spanish Institute of Oceanography (IEO), Regional Government of Andalusia, Coastal Ecology Institute Foundation **Malta:** Malta Center for Fisheries Science (MCFS) **Cyprus:** Department of Fisheries and Marine Research, Ministry of Agriculture (DFMR) **Croatia:** Institute of Oceanography and Fisheries (IOR) **Greece:** Hellenic Center for Marine Research (HCMR), Special Secretariat for Water, Ministry of Environment **Turkey:** Ministry of Environment and Urbanization, Ministry of Forestry and Water Affairs **France:** National Center for Marine Sciences (CNRS) **Morocco:** National Institute of Fisheries Research (INRH) **Israel:** Israel electric cororation (IEC) **Slovenia**: National Institute of Biology (NIB) **Montenegro:** Public Enterprise for Coastal Zone Management (Morsko dobro)	The Mediterranean Science Commission (CIESM) **France:** Wetlands International **Malta:** International Ocean Institute **Italy:** long-term Ecological Research Network (LTER Italia) **Turkey:** Scientific and Technological Research Council of Turkey-Marmara Research Center (TUBITAK-MAM)
BLACK SEA					
Habitats Directive, Birds Directive, Water Framework Directive (WFD), Marine Strategy Framework Directive (MSFD), Common Fisheries Policy (CFP)	Black Sea Integrated Monitoring and Assessment Program (BSIMAP), Black Sea Strategic Action Plan (BSSAP)	Convention on the Protection of the Black Sea Against Pollution (Bucharest Convention)	**Ukraine:** Program for the environmental monitoring of the Black and Azov Seas (MEM)	**Russia:** Russian Academy of Science, Ministry of Natural resources and environment **Bulgaria:** Academy of Sciences (IO-BAS), Ministry of Environment and Water (MOEW) **Romania:** National Institute for Marine Research and Development (NIMRD), National Agency for Fisheries and Aquaculture (NAFA) **Ukraine:** National Academy of Science of Ukraine, Ministry of Ecology and Natural Resources **Turkey:** Ministry of Environment and Urbanization, Ministry of Forestry and Water Affairs	The Mediterranean Science Commission (CIESM) **Bulgaria:** Society for the Protection of Birds (BSPB), AES GEO Energy **Romania:** MARE Nostrum NGO **Georgia:** Black Sea Monitoring Division, Batumi State University **Russia:** Shirshov Institute of Oceanology, A.O.Kovalevsky Institute of Marine Biological Research **Ukraine:** Ukrainian Scientific Center of Ecology of Sea (UkrSCES), Institute of Marine Biology of NASU. **Turkey:** Scientific and Technological Research Council of Turkey-Marmara Research Center (TUBITAK-MAM)

Parties which undertake these monitoring programs are identified. European Directives are common to all regions and EU Member States, but may not be listed in all regions as these are not always specifically and uniquely linked to reported monitoring programs (i.e., monitoring data to fulfill these objectives may be amalgamated from a number of programs).

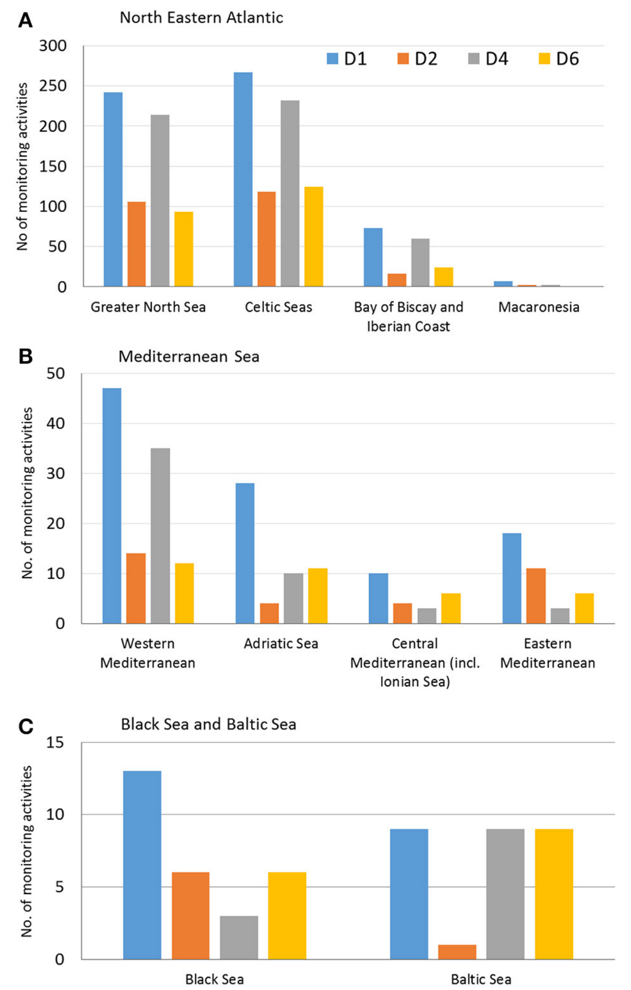

FIGURE 4 | Number of monitoring activities per MSFD Descriptor. D1, biological diversity, D2, non-indigenous species; D4, food webs; D6, seafloor integrity, in **(A)**. the North Eastern Atlantic sub-regions, **(B)**. Mediterranean Sea sub-regions and **(C)**. Black Sea and Baltic Sea.

monitoring for deep sea fish, deep sea elasmobranchs, and ice-associated fish lacking or limited to a small number of programs. This pattern is mirrored in the corresponding habitats which lack or have limited monitoring (i.e., deep sea and ice-associated habitats). In addition, most of the fish monitoring focuses on commercial species and less on non-commercial species or is focused on the fish in transitional waters (e.g., estuaries, fjords) as required by the WFD. The limited monitoring for reptiles, mammals, and birds in most Regional Seas was not expected since such monitoring is required in the Habitats and Birds Directives. The same applies to the identified gaps in cephalopod monitoring, expected to be already operational for the Common Fisheries Policy (CFP). Whilst these gaps could be due to incomplete reporting, they may indicate that the implementation of the EU environmental and fisheries related *acquis* has been limited. However, since some of these components (e.g., mammals) are indeed monitored under the Habitats Directive and regular status updates (every 6 or more years) are freely available through the Article 17 portal for that Directive, it is the lack of access to the monitoring information that represents a problem.

Monitoring programs that address microbes are limited in the NEA and Mediterranean Sea or lacking in the Black Sea (**Figure 5**). With the exception of microbes, biodiversity components that belong to low trophic levels are generally well addressed by monitoring programs in all Regional Seas, however with a smaller number of offshore stations in all relevant components compared to coastal stations, particularly in the Baltic Sea. Zooplankton monitoring also appears limited in the Mediterranean Adriatic and Central Mediterranean Sea. The lack of microbial diversity monitoring is expected as, with the exception of pathogens in the Bathing Water Directive, it was not previously addressed at the European level. Nevertheless, the overall rather good coverage of low trophic level monitoring could be related to the long European tradition of eutrophication monitoring and to the similar requirements of monitoring eutrophication under the WFD (Ferreira et al., 2011). Similarly, and against a declining trend in monitoring effort, de Jonge et al. (2006) emphasized both the lack on monitoring on these lower trophic components and the lack of monitoring on functioning rather than on structure in marine systems.

Quality Assurance (QA) and Supporting Physicochemical Data

For a number of biodiversity components QA is lacking. The BEQUALM (Biological Effects Quality Assurance in Monitoring Programmes) and UK NMBAQC (National Marine Biological Analytical Quality Control) schemes respectively for contaminants and benthic invertebrates do provide Analytical Quality Control and QA (Gray and Elliott, 2009) in some Regional Seas (e.g., NEA and Black Sea). However, approximately half of the monitoring activities do not collect supporting physicochemical data which thus provides a major drawback in having sufficient information to explain the ecological findings.

Habitats

With respect to the seabed and water column, most monitoring activities have been reported to cover "others" instead of a specific habitat from the list. This indicates that these activities cover several habitats and in many instances notes were added including coverage in multiple habitats. The monitoring activities that cover a specific seabed habitat are most numerous for "littoral sediment" in the NEA, and Mediterranean and Black Seas. In total, 10 seabed habitats have not been reported to be covered by monitoring activities. Nevertheless, these habitats might be covered by the monitoring activities which have been reported to cover "others" (i.e., 256 activities in the NEA, 22 in the Mediterranean and four in the Black Sea). In the water column, the NEA monitoring activities cover all five habitats and the Mediterranean activities cover four habitats (i.e., "variable salinity (estuarine) water" is not covered). In the Black Sea only "marine water: coastal" and "marine water: shelf" are indicated to be covered by monitoring activities but these may be regarded as "catch-all" terms. As with seabed habitats, the water column habitats which do not seem to be covered could be monitored

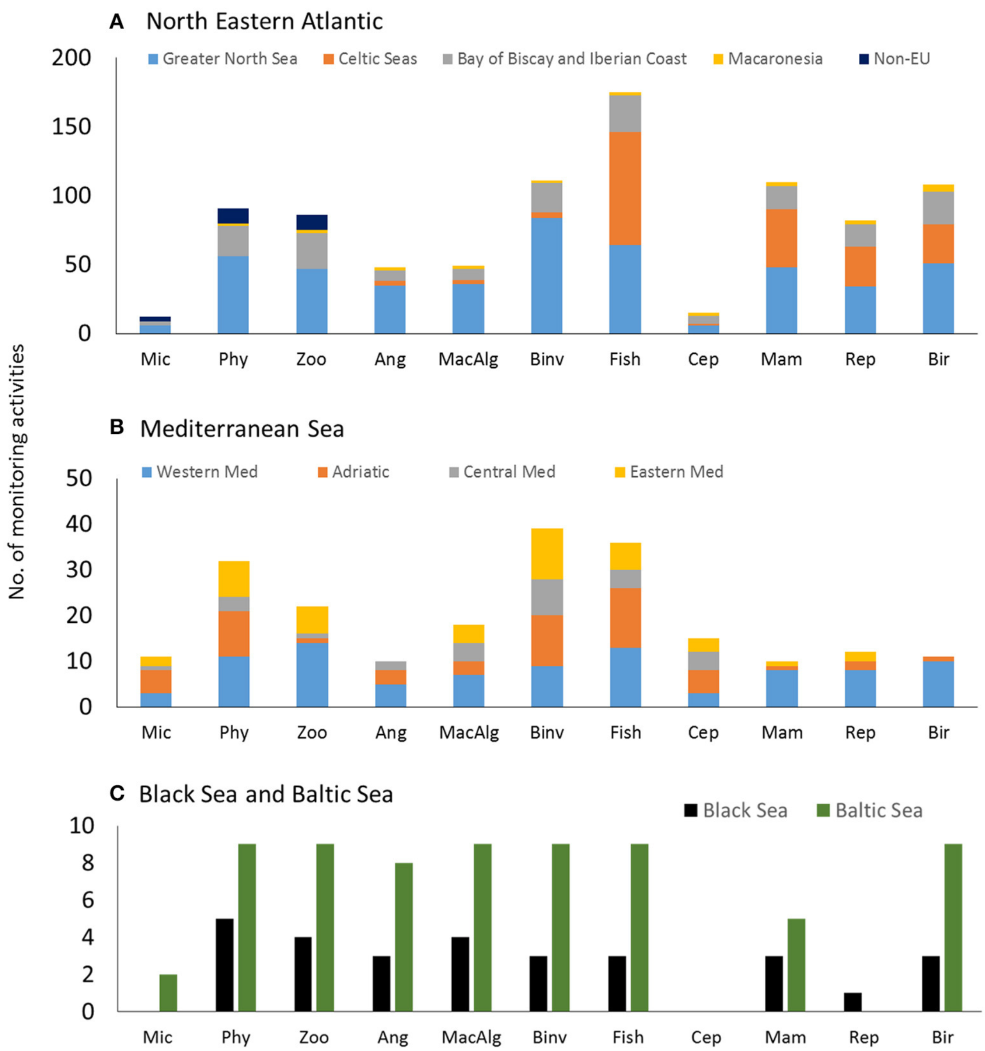

FIGURE 5 | Number of monitoring activities per biological component in (A). The North Eastern Atlantic sub-regions, **(B)**. Mediterranean Sea sub-regions and **(C)**. Black Sea and Baltic Sea. Mic, microbes; Phy, phytoplankton; Zoo, zooplankton; Ang, angiosperms; MacAlg, macroalgae; Binv, benthic invertebrates; Cep, cephalopods; Mam, marine mammals; Rep, marine reptiles; Bir, marine birds; Western Med, Western Mediterranean; Central Med, Central Mediterranean including the Ionian Sea; Eastern Med, Eastern Mediterranean.

through activities that include "others" (i.e., 383 in the NEA, 32 in the Mediterranean and three in the Black Sea), however, as stated above this could not be verified. Monitoring programs addressing ice-associated habitats are recorded as completely lacking on those Regional Seas where these habitats occur (NEA and Baltic), which could be partially attributed to the monitoring activities targeting this habitat indirectly through monitoring focusing in the ice-associated species or communities (e.g., seals; Teixeira et al., 2014), but also to a lack of input from more Northern countries.

Pressures

In the Greater North Sea and Celtic Sea (NEA), all 37 pressures are covered by monitoring activities (**Figure 6A**). In the Baltic Sea, 26 pressures are covered. Although there are between 11 and 25 pressures covered in the Mediterranean, in the Black Sea, and in the NEA sub-regions Bay of Biscay and Iberian Coast and Macaronesia, the actual number of monitoring activities covering these pressures is limited when compared to the Greater North Sea and Celtic Sea (North Eastern Atlantic; **Figure 6A**).

Despite it being an individual MSFD descriptor (D11–introduction of energy), monitoring programs addressing the pressure "underwater noise" are limited in the Baltic Sea and Black Sea (i.e., only one monitoring activity reported) and lacking in the Bay of Biscay and Iberian Coast and Macaronesia (both NEA) and the Mediterranean (**Figure 6B**). At present, the impact of noise on many biodiversity components is not well understood (e.g., Roberts et al., 2015) and the outputs of such monitoring cannot be used effectively. Also the pressures "marine litter," "noise," and "introduction of non-indigenous species" are mainly monitored in the NEA and coverage is limited in other regional seas. The limitation in monitoring activities for the first

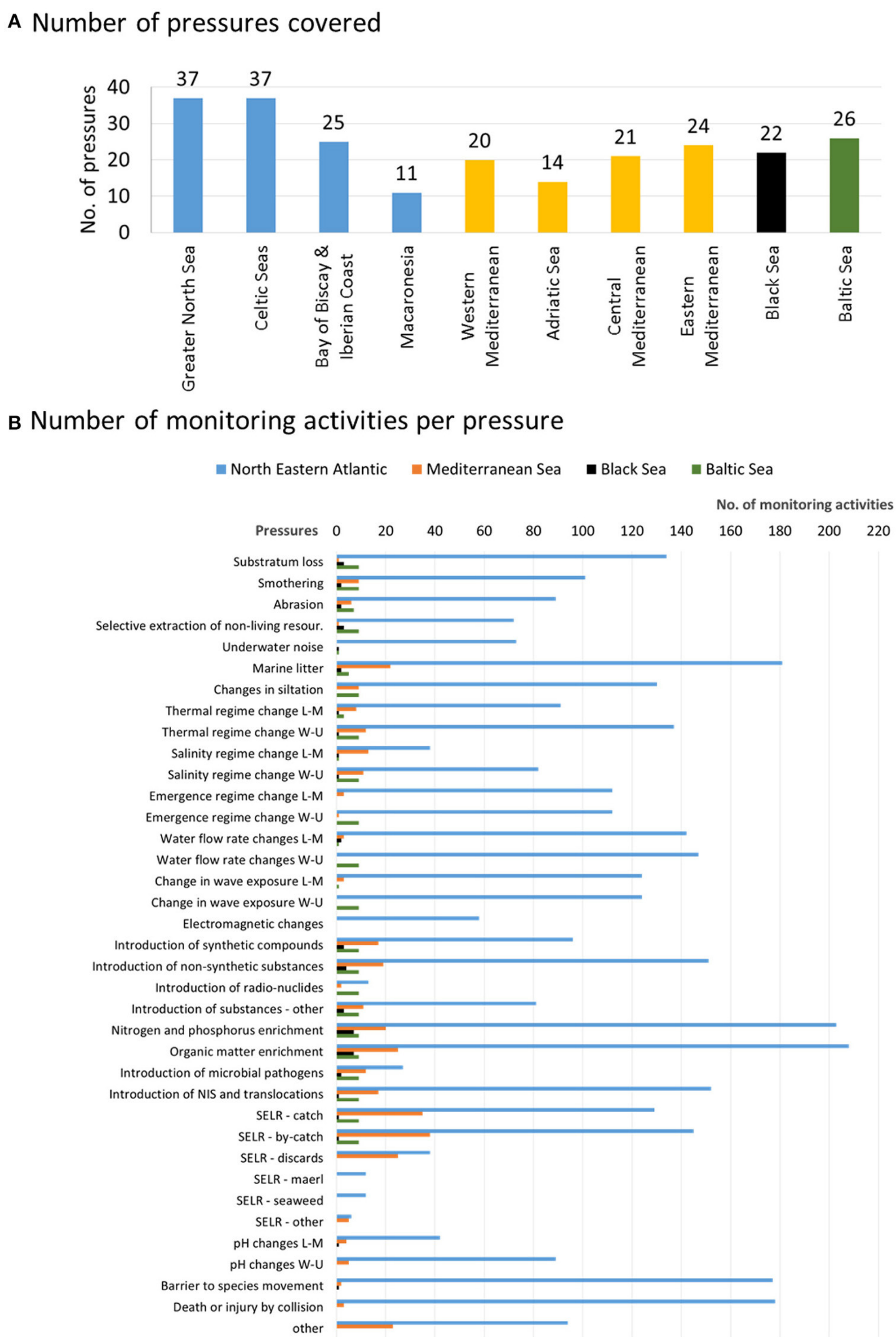

FIGURE 6 | (A) Number of pressures covered and **(B)**. Number of monitoring activities per pressure in the North Eastern Atlantic (sub-regions: Greater North Sea, Celtic Seas, Bay of Biscay, and Iberian Coast, Macaronesia), Mediterranean Sea (sub-regions: Western Mediterranean, Adriatic Sea, Central Mediterranean including the Ionian Sea, Eastern Mediterranean), Black Sea, and Baltic Sea. L-M, Local and manageable; W-U, Widespread and unmanageable; SELR, Selective extraction of living resources.

two of these pressures in the catalog represents a partial gap as they are directly linked to MSFD descriptors not targeted by this catalog (i.e., D10–marine litter and D11–introduction of energy). In the Baltic Sea, until systematic non-indigenous species (NIS) monitoring programs (Lehtiniemi et al., 2015) and port biological sampling (HELCOM, 2013) are routinely established with wider Baltic coverage, the primary sources for NIS occurrence, their distribution and population size estimates remain non-systematic and include "inherent uncertainty" as this information depends on data collection for other purposes than NIS surveillance. Therefore, one of the major issues still to be solved is the establishment of an internationally coordinated monitoring system for NIS/Cryptogenic Species in the Baltic Sea and in other areas (Olenin et al., 2011). However, because of the high degree of concern regarding NIS emanating from the Suez Canal into the Mediterranean Sea, then this has resulted in more information available for parts of the Mediterranean Sea (Galil et al., 2014).

Monitoring programs addressing the pressures "water flow rate changes (widespread-unmanageable)," "change in wave exposure (widespread-unmanageable)," and "electromagnetic changes" are also lacking in the Black Sea and the Mediterranean Sea. Similarly, the pressure "introduction of radionuclides" is generally limited or lacking in all regional seas although this is incorporated into compliance monitoring (as conditions under their license to operate) carried out by nuclear power and reprocessing authorities and industries.

Monitoring for the "selective extraction of living resources," the pressures "catch," "bycatch," and "discards" is covered in the NEA, Baltic Sea and Mediterranean Sea, but lacking or limited in the Black Sea. The coverage of these pressures could be due to the fact that they are also being monitored through the EU Common Fisheries Policy and Data Collection Framework. Activities monitoring the pressures "maerl extraction" and "seaweed extraction" are limited in the NEA and lacking in the Mediterranean and Black Sea (there is limited commercial extraction and production in those areas).

What Are the Strengths and Weaknesses of the Existing Marine Biodiversity Monitoring in Europe?

Strengths

As indicated above, there is a long history of monitoring in the European Regional Seas which has enabled the standardization of techniques and the development of best practice. For example, in the NEA and the Baltic Sea, monitoring starts from the early 1900s and in all Regional Seas at least some monitoring has taken place since the 1950s, with the number of programs increasing to the present day. Monitoring started to become more coordinated in the 1970s with the formation of HELCOM for the Baltic Sea and the Oslo and Paris Conventions (now OSPAR) for the NEA. Within each Regional Sea, it is generally common practice to collect supporting physico-chemical data simultaneously with biological data in order to explain biological change and several programs have associate formal QA guidelines to ensure validity of the data. Furthermore, for the four MSFD descriptors considered, all biodiversity components, habitats and pressures are addressed to some extent in all Regional Seas, with some programs addressing multiple descriptors. This provides a strong basis for the implementation of the MSFD and the assessment of GES. In most Regional Seas, the 11 biodiversity components are being covered and several are monitored simultaneously. Similarly, most monitoring programs concurrently address more than one seabed and water column habitat, thus optimizing the sampling efforts and providing an holistic approach to environmental monitoring. In general, most monitoring programs address more than one pressure. Although these are exceptions, some monitoring activities assess 18–20 pressures simultaneously (e.g., Celtic Sea sub-region), suggesting the potential for monitoring programs to become more efficient.

Weaknesses

Whilst the information in the catalog has enabled a broad spatial and temporal assessment of monitoring throughout Europe, it cannot be used to assess completely the adequacy of monitoring although it does identify areas which require further development. For example, whilst it is apparent that all descriptors, habitats and biodiversity components are being addressed, this is only the case for certain areas of some Regional Seas (e.g., in the territorial waters of a single nation). Detailed analysis at the individual Regional Sea level highlights this uneven distribution of monitoring activities at a spatial (sampling sites and stations) and temporal (sampling interval and frequency) level. Additionally, in a number of sub-regions, marine biodiversity monitoring programs address a specific target only (e.g., a particular habitat, species, pressure, etc.) resulting in an uneven distribution of monitored components (i.e., not all components are monitored in all sub-regions). For example, the NEA sub-regions Greater North Sea and Celtic Sea have the most reported monitoring activities of all Regional Seas; in contrast the NEA sub-region Macaronesia has a limited number of monitoring activities and contains several major gaps (e.g., no monitoring activities of D2 and D6). This may be partially an artifact of an incomplete coverage of the catalog, but it still reflects significant imbalances. It may also reflect the fact that monitoring historically has been driven by the presence or societal perception of problems, i.e., if society considers there to be an environmental problem then the authorities are more likely to respond and similarly pristine areas are not deemed to require extensive (if at all) monitoring (de Jonge et al., 2006).

In this broad-scale assessment, the number of monitoring programs that simultaneously address biodiversity components, descriptors, habitats and pressures (managed and unmanaged) is used in our study as a measure of the robustness of ongoing monitoring to potentially meet the requirements of the MSFD (to achieve GES) in all Regional Seas. However, whilst there is much information indicating the presence/absence of supporting physico-chemical data and QA to support this, detailed information on sampling design, sampling frequency, methodology, and the status of the QA programs (e.g., is it a national/international programs which includes assessment of the performance of participants?) is required to assess whether or not the monitoring is fit-for-purpose. Indeed, whilst monitoring in some areas is well developed, the associated indicators for the MSFD assessment of some descriptors are still under development indicating a weakness that needs to be addressed before the requirements of the MSFD can be fully met (Teixeira et al., 2014). Integrated monitoring is more likely to capture intricate ecological relationships, while at the same time the identification of anthropogenic cascade effects and cumulative or in-combination effects may be better identified if monitoring is coordinated in time and space. This includes bottom-up processes and top-down responses, and thus an analysis of ecosystem functioning as well as ecosystem structure, which underpins the Ecosystem-based approach, a central pillar of the MSFD and marine management (Elliott, 2014). Several monitoring programs both within and between regional seas address single or a limited number of components, habitats and pressures and although not explicitly investigated within the catalog, may be limited in terms of spatial (e.g., geographic area, sampling locations) and temporal (time-series, sampling frequency) scale. There is a need for more efficient and robust monitoring programs, integrating several biodiversity components, habitats and pressures through simultaneous monitoring, especially where pressures emanate through the whole ecosystem. Additionally, despite the extensive system of monitoring programs in most Regional Seas, a number of biodiversity components (e.g., microbes), descriptors (e.g., NIS), habitats (e.g., ice or deep sea habitats) and pressures (e.g., noise, introduction of radionuclides, selective extraction of living resources such as seaweed and maerl) are poorly or not addressed. Furthermore, most monitoring is focused on ecosystem structural aspects (the number of species, size of population, cover by a species) rather than on functional aspects (rate processes) even though the MSFD may change this emphasis (Borja et al., 2010; Hering et al., 2010).

The weaknesses identified are not trivial, as they concern some of the most relevant and elemental attributes of sound biodiversity monitoring schemes, recently identified by Pocock et al. (2015), for example, articulate objectives, standardized methodology, suitable field sampling methods, taxonomic literature, national, or regional coordination, data entry systems, QA of data, or/and scientific sampling design. Similarly, monitoring has to provide the 18 attributes for creating sound indicators and monitored elements given by Elliott (2011). Nevertheless, these findings can be used to reassess priorities when planning development or adjustment of the biodiversity monitoring programs in the future.

What Are the Threats and Opportunities of the Existing Marine Biodiversity Monitoring in Europe?

Threats

Budgetary constraints are the most significant and obvious threat to monitoring within EU Member States (e.g., Borja and Elliott, 2013) thus giving rise to what has been termed the "monitoring requirement paradox," that there is an increasing amount of governance requiring monitoring while at the same time monitoring budgets have been cut (Borja et al., 2016; Strong and Elliott, accepted). For example, even where monitoring is undertaken within networks with standardized protocols (e.g., MEDITS and MEDPOL), budgetary constraints can result in countries suffering from data gaps over several years (see also de Jonge et al., 2006).

As identified above, achieving GES through the implementation of the MSFD is only attainable if the current and future monitoring of marine biodiversity is improved in all European Regional Seas. The number of ecosystem components monitored needs to be increased and specific monitoring programs developed to analyze pressures and pressure-impact relationships (Scharin et al., 2016). It may also be necessary to standardize sampling methods, increase sampling frequency and intensify sampling design in some regional seas. In order to ensure successful integrative monitoring schemes within and between Regional Seas, it may be necessary to establish a sustainable funding scheme and/or research budget and a rapid response/intervention framework. In the current economic climate it is difficult to envisage that EU Member States would be able to provide an appropriate budget for this but at present there is no pan-European or EU mechanism for funding monitoring across Member States. It is likely to remain the case that funding within a given area is the responsibility of that Member State.

The integration and holistic assessment of monitoring data at the Regional Sea level may be difficult, time consuming and economically restrictive due to methodological differences between EU Member States. This is also partly due to some EU Member States having a long history of monitoring and where many programs have been expanded, modified and developed over time. Hence, rather than establish new monitoring programs which specifically address MSFD objectives, EU Member States may rely on existing programs, which may be inadequate or not suitable, particularly where these have been designed for other purposes. Hence, when required to submit their MSFD monitoring proposals in 2014, EU Member States appear to report what they were doing rather than what they were required to do additionally for the directive (Boyes and Elliott, 2014), an approach which is expected to lead to anomalies and gaps (Boyes et al., 2016). In addition, differences in methods between countries which then need to produce a uniform assessment, will then need inter-calibration and inter-comparison exercises as has been carried out during the implementation of the EU WFD (e.g., Hering et al., 2010; Lepage et al., 2016).

Regional cooperation is required between EU Member and Non-Member States to implement the MSFD (Cavallo et al., 2016), although Non-Member States are under no legislative requirement to achieve GES in their respective regional seas. However, sea areas controlled by a combination of Member States and Non-Member States will still require coordination to tackle transboundary problems; this will certainly be the case for any current Member State which leaves the EU (Boyes and Elliott, 2016). If agreements with Non-Member States are not in place, achieving this cooperation may put undue additional pressure on EU Member States and may mean that infractions (proceedings in the European Court that a Member State has failed to meet a Directive) cannot be prosecuted and GES in the Regional Sea may not be achieved. For example, Norway is a non-Member State of the EU therefore not implementing the MSFD but it is still performing many of the aspects required by the Directive as well as being a leading member of OSPAR and following its monitoring and assessment protocols. Accordingly Regional Sea Conventions have an important role in this coordination, for example through the OSPAR, HELCOM, and UNEP/MAP monitoring and assessment programs.

Opportunities

Several inadequacies have been identified in the monitoring currently undertaken in the Regional Seas. This presents a number of opportunities to modify and/or expand existing monitoring programs, develop new programs and to collaborate between EU Member States to develop standardized and robust programs and networks. These can occur both within and between Regional Seas that maximize the use of the best available data.

This would mean, for example, standardized verification of analyses and species identification, inter-calibration exercises for hazardous substance concentrations in biota, introduction and/or integration of validated external QA protocols, and a focus on upgrading the spatial and temporal resolution of monitoring and inter-calibration procedures. Introducing the simultaneous monitoring of descriptors, biodiversity components, habitats and pressures within single, large monitoring programs and ensuring that monitoring is designed to address specific pressures would increase the robustness of monitoring. This may also give an opportunity to create an online bank of all monitoring program data, accessible to all EU Member States, which should include information collected under different Directives and research programs (e.g., CFP, WFD, MSFD, EU funded projects, etc.). Creating such a uniform data storage system is being accomplished both at an EU scale, e.g., through the European Environment Agency and EMODnet (http://www.emodnet.eu/), and at an EU Member State level such as MEDIN in the UK (see http://www.oceannet.org/).

Those EU Member States that are members of Regional Sea Conventions (RSC) with a long history of marine monitoring and assessment, such as OSPAR and HELCOM, which have had joint monitoring programs since the 1970s, can provide valuable experience to states with a lesser history. The opportunity for collaborative work afforded by the implementation of the MSFD enables EU Member and Non-Member States to improve and/or develop monitoring programs to achieve GES in some regional seas (i.e., the Black Sea, Mediterranean Sea). This regional cooperation may prove essential for achieving GES in Regional Seas that border non-EU nations. For example, non-EU Member States of the Black Sea and Mediterranean Sea, respectively, should be encouraged through the Black Sea Commission and UNEP/MAP to develop more integrated monitoring programs (especially for the descriptors related to biodiversity monitoring). However, costs associated with activities required by RSC are borne by the country and so each country is required to fund its own commitments. Despite this, funding is becoming increasingly available from the EU, for example, to develop Integrated Regional Monitoring Implementation Strategies in the Mediterranean and Black Seas and basin-wide promotion of MSFD principles (PERSEUS and IRIS-SES projects). However, funding the development of strategies and principles may not be the same as funding the monitoring. Accordingly, our findings regarding the inadequacies in the monitoring currently undertaken in the European regional seas form the basis of further research proposals and requirements.

Conclusions and Recommendations to Overcome the Identified Impediments and Develop More Robust Monitoring Strategies for the Future

The MSFD explicitly spells out that the assessment strategy is to be implemented at the regional or sub-regional level with both the individual EU Member States and, whenever possible, third countries (sharing the regions/sub-regions) acting together coherently and in a coordinated fashion through regional institutional cooperation structures. The success of the MSFD depends on a high level of cooperation between EU Member States, third countries and regional bodies mandated with environmental protection responsibilities (Long, 2011; Cavallo et al., 2016). Monitoring programs are to be compatible within marine regions or sub-regions and monitoring methods are to be consistent so as to facilitate comparability of monitoring results (Karydis and Kitsiou, 2013). The MSFD further specifies that standardized methods for monitoring and assessment be adopted (Zampoukas et al., 2013) thus putting the onus on the activities of the EU Member States, through coordination by the Regional Sea Conventions and even between RSC. Although there is some detail as to the descriptors or types of biological and other components that should be monitored, given that the MSFD is a Framework Directive, then the method of monitoring is left to the EU Member State level. This can create a large variation and incompatibility between, for example, two EU Member States that share marine borders within the same Regional Sea. There has always been a North and West compared to South and East difference within Europe with the former areas having more developed regional governance and organization, more detailed and long-standing administrative/legislative frameworks, a longer history and culture of environmental management and greater resources. A complicating feature is in the make-up of the Regional Seas, where in northern Regional Sea areas EU Member States comprise more than 80% of the participants compared to the Mediterranean and Black Sea regional areas where EU Member States make up less than 40% of the participant states. In the latter cases therefore, reaching GES for the whole region would require substantive support from the non-EU Member States, the relevant RSC and the EU Member States. The northern RSC (HELCOM and OSPAR) have a much longer experience of coordinated monitoring than the southern ones (UNEP/MAP, Black Sea Commission) and the western Member States have a longer history of compliance with EU environmental Directives than the eastern states. Hence, as all Member States have to implement and comply with the Directives then the intent of the MSFD needs to be reinforced to provide a much stronger level of clear coordination and standardization in the southerly Regional Seas (Zampoukas et al., 2013).

We acknowledge that the database on which the analysis here is based has some omissions and that the regional and national monitoring effort is changing annually. Despite that, we consider that the major and general lessons learned from its interrogation are robust and will hold even for a more complete database. As such, we strongly recommend the following:

1. Specific coordination within the Regional Sea MSFD monitoring programs—this will require specific Regional Sea committees/representation/meetings with EU Member States and non-Member States to discuss, agree and set up detailed Regional Sea recommendations, guidelines and specific implementation plans. In turn, this will allow for the growth of large, coordinated datasets for the (sub)regions of each Regional Sea, and, in a near future, will allow establishing a common platform for data sharing (resulting from different Directives and EU policies, e.g., EMODnet) that should be compulsory for all EU Member States.
2. Standardization of methodologies, based on the follow-on from Regional Sea meeting workshops, on selection, set-up of protocols and training, for:
   a) Choice of indicators for each of the descriptors, including coverage of under-monitored components (e.g., microbes) and to monitor functional as well as structural aspects;
   b) Developing methods which can cover large sea areas more efficiently (e.g., landers, gliders and seabed scanning) and provide the surveillance monitoring against which future investigative and diagnostic monitoring is carried out when marine environmental adverse effects are detected;
   c) Design of spatial and temporal coverage for indicator measurement (including replication) which includes reconciling the compromise between monitoring effort and the capability of detecting impacts;
   d) More effective methodologies for sampling, sample processing and analysis to produce data for the selected indicators (or proxies for those indicators), and
   e) Quality assurance/control of the sampling and analytical process and using inter-comparison and inter-calibration exercises where necessary and where possible.
3. EU Member States to specifically budget for sufficient monitoring and coordination activities but ensure these are cost-effective. Borja and Elliott (2013) have noted that the consequence of the choices made now, during times of economic crisis, increases the possibility that European countries will not produce useful information for management. To avoid this and to maximize information gained relative to resources required for data collection and analysis, Levine et al. (2014) and Franco et al. (2015) suggest that sampling designs should be established to account for uncertainty analyses, thus improving the efficiency of environmental monitoring. Such designs would have to be statistically based, perhaps using power analysis, against the required detection limits and thresholds of effect (Franco et al., 2015). There is a mismatch between Member States concerning size of area of marine responsibility and their existing capabilities/resources and at present there appears to be no facility for centralized EU funding for monitoring nor for the transfer of monitoring funds across Member States from those with better resources to less-advantaged Member States. However, Member States can cooperate and one can even carry out monitoring on behalf of another.

Monitoring programs under the MSFD must be compatible with assessment obligations arising from other regional and EU or international instruments for reasons of continuity and efficiency (Long, 2011). While it is easy for an EU Member State to adapt or extend an existing monitoring program, they need to be fit-for-purpose with at least the minimum requirement to ensure adequate, defendable and meaningful assessments. Given that there is likely to be an increasing litigious framework, where assessments may be challenged legally in infraction proceedings (e.g., see Elliott et al., 2015), then the monitoring and resultant data have to be robust to those challenges.

As some of the descriptors or components may be new to established traditional monitoring [e.g., D2–NIS (e.g., see Olenin et al., 2011) and D11—introduction of energy—underwater noise (e.g., Roberts et al., 2015)] or as trends move from structural to functional ecosystem aspects (e.g., Strong et al., 2015), there is the need to develop/adopt cost-effective and innovative methods for monitoring including both state-of-the-art methods/tools and citizen science. Complementing existing monitoring programs for example for the EU WFD to explicitly deal with gap-filling on invasive species is recommended (Zampoukas et al., 2014) as well as integrating assessments made under the Habitats and Birds Directives for mammals, reptiles and birds.

Although, as shown here, there is a good basis on which to build, several EU Member States will need to broaden the scope and expand monitoring coverage and intensity to comprehensively assess the environmental status of their waters. Integrated monitoring programs taking into account a common vision on operational objectives and on indicators and targets for GES, are needed to achieve and maintain a particular or minimum desired level of environmental quality (Cinnirella et al., 2012) at the regional level. In addition, as the protection of the environment and the conservation of marine ecosystems functioning are now rooted in the EU regulatory code as binding legal obligations (Long, 2011; Boyes and Elliott, 2014), standards and protocols also need to be enacted to make the assessments strong, robust and legally defensible if challenged. It is emphasized and acknowledged that while many of the recommendations given here require better, more extensive and perhaps more costly monitoring, this is required to avoid any legal challenges to the assessments or to bodies and industries accused of causing a deterioration in marine quality.

Finally, it is emphasized that the detection of GES rest wholly on the adequacy of monitoring and the ability to detect a signal of change against a background of inherent variability, and conversely that inadequate monitoring will not be able to determine either such a change or determine whether management measures have had the desired effect.

AUTHOR CONTRIBUTIONS

JP led the work on the manuscript. JP, SL, KM, KP, CS, HH compiled data, analyzed data (status, gaps, and SWOT), conceived, structured and wrote the manuscript. JP and HH constructed the figures. HT and KM built the catalog, compiled data, wrote the manuscript. MU, OS, AZ, GK, OK, TC, SM, MB collected data to fill in the catalog, did the regional data analysis and status/gaps/SWOT and contributed to the manuscript. AB, NH contributed to and revised the manuscript. ME envisioned the need for the monitoring catalog, wrote and revised the manuscript.

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Evaluation of Alternative High-Throughput Sequencing Methodologies for the Monitoring of Marine Picoplanktonic Biodiversity Based on rRNA Gene Amplicons

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Sequencing of rRNA gene polymerase chain reaction amplicons (rRNA tags) is the most common approach for investigating microbial diversity. The recent development of high-throughput sequencing (HTS) technologies has enabled the exploration of microbial biodiversity at an unprecedented scale, greatly expanding our knowledge on the microbiomes of marine ecosystems. These approaches provide accurate, fast, and cost efficient observations of the marine communities, and thus, may be suitable tools in biodiversity monitoring programs. To reach this goal, consistent and comparable methodologies must be used over time and within sites. Here, we have performed a cross-platform study of the two most common HTS methodologies, i.e., 454-pyrosequencing and Illumina tags to evaluate their usefulness in biodiversity monitoring and assessment of environmental status. Picoplankton biodiversity has been compared through both methodologies by sequencing the 16 and 18 S rRNA genes of a set of samples collected in the coast of Barcelona (NW Mediterranean). The results show that, despite differences observed in the rare OTUs retrieved, both platforms provide a comparable view of the marine picoplankton communities. On a taxonomic level, there was an accurate overlap in the detected phyla between the two methods and the overall estimates of alpha- and betadiversity were comparable. In addition, we explored the concept of "indicator species" and found that certain taxa (i.e., members of the Gammaproteobacteria among others) as well as the ratio between some phylogenetic groups (i.e., the ratio of Alphaproteobacteria/Gammaproteobacteria, *Alteromonas*/SAR11, and *Alteromonas* + Oceanospirillales/SAR11) have potential for being useful indicators of environmental status. The data show that implementing new protocols and identifying indicators of environmental status based on rRNA amplicon sequencing is feasible, and that is worth exploring whether the identified indices are universally applicable.

Keywords: plankton diversity, high-throughput sequencing, marine ecosystems, prokaryotes, picoplankton, monitoring programs, indicators, environmental status

INTRODUCTION

The oceans are the largest ecosystem on Earth and provide countless ecosystem services to society (Liquete et al., 2013). Oceans regulate our planet's climate and represent one of the largest carbon reservoirs in the globe. Over a third of the world's population live in coastal areas, but virtually all humans depend to some extent on the ocean. Marine ecosystems provide resources for human survival and well-being, from fishing to natural products used in medicine or biotechnology. However, human-impacted marine ecosystems (i.e., coastal areas) are being increasingly threatened by pressures exerted due to changes in land use, overfishing, climate change, pollution, aquaculture, invasive species and other impacts of a rapidly growing human population (Halpern et al., 2007, 2008). Therefore, there is a need to report on the condition of the marine ecosystem in response to these human pressures, which may have an effect on all the components of the marine food web, from microorganisms to top animal predators (Brown et al., 2010; Claudet and Fraschetti, 2010; Hoegh-Guldberg and Bruno, 2010) and in the ocean services.

Legislation regarding the management of human impacts on the marine environment has been implemented worldwide to protect and conserve marine ecosystems. Several international (United Nations Convention on the Law of the Sea), regional [i.e., Marine Strategy Framework Directive (MSFD) in Europe, Oceans Act in the USA (Birk et al., 2012) among others] and local initiatives to protect the oceans exist. These initiatives include a number of criteria and methodological standards for assessing the environmental status of marine waters. The effect of anthropogenic impacts on the marine ecosystem is currently assessed through a variety of approaches (Birk et al., 2012). In any marine environmental assessment carried out for legislative or non-legislative reasons, there is a need to develop and test indicators at the species, habitat and ecosystem level. There is also a need for the cost-effective implementation of these indicators by defining monitoring and assessment strategies that are as simple, fast, and cheap as possible.

Among the different biological components of the marine ecosystem potentially used as indicators, the least known are the microbial communities, which are the major contributors to global marine diversity, and are a dominant component of the whole aquatic biota in terms of biomass and activity. Furthermore, they play a crucial role in its contribution to primary production and processing of organic matter (Kirchman, 2008). Microorganisms are the smallest biotic components and their intrinsic growth rates are the fastest among all biological components of natural aquatic systems. Microbial communities increase cell numbers as response to nutrients inputs, and as a consequence decrease their diversity, which also occurs in response to events of acute contamination (see review by Nogales et al., 2011). Since microorganisms are the fastest biotic responders to environmental changes, their abundances, community composition (i.e., the taxa present and their relative abundances) and relative indications of their activity have the potential for becoming useful indicators of ecosystem condition. Indeed, microbial indicators have been proposed in several legislative directives, such as the MSFD descriptors of biodiversity, food webs, eutrophication, and seafood contaminants. Including microbes in future monitoring programs has already been suggested (Caruso et al., 2015), and an intense research on this direction is being carried out particularly since the introduction of genetic methodologies.

Genetic technologies have the potential to provide accurate, rapid, and cost efficient observations of the marine environment. Molecular methods also represent a reliable taxonomic identification tool especially for organisms lacking conspicuous morphological traits such as microorganisms. Several molecular methods have been proposed for integration into existing monitoring programs (e.g., qPCR, SNP based methods, DNA barcoding, microarrays, metagenomics, metatranscriptomics; see review by Bourlat et al., 2013). Among those, DNA tagging (i.e., DNA barcoding or assigning taxonomy to a specimen/sample by sequencing a short DNA fragment) has a high potential for marine monitoring and assessment because of its relatively low cost and easy standardization once a reference database has been built.

The recent development of high-throughput sequencing (HTS) technologies has enabled the exploration of microbial biodiversity at an unprecedented scale, greatly expanding our knowledge on the microbiomes of different ecosystems (Cho and Blaser, 2012; Gilbert et al., 2014) including the oceans (Ferrera et al., 2015; Moran, 2015). Sequencing of rRNA gene polymerase chain reaction amplicons (rRNA tags) is currently the most common approach for investigating microbial biodiversity. Because this approach provides accurate, fast and cost efficient observations of the marine environment, it may be a suitable tool in biodiversity monitoring programs. While the potential for this method exists, testing and pilot studies are needed to answer relevant questions, for example, their benefits as compared to more traditional methods, and to test their general applicability (Bourlat et al., 2013).

In this study we evaluated two of the most commonly used HTS methodologies, i.e., 454-pyrosequencing (from now on 454) and Illumina, to study marine picoplanktonic biodiversity and explored their use in the assessment of ecosystem health status. The 454 method has been the most popular methodology since the development of HTS as it was the first to become commercially available and offers relatively long read length. The International Census of Marine Microbes program (Huse et al., 2008) used this approach. In contrast, Illumina provides shorter reads but offers significantly greater throughput than 454 at lower cost (Glenn, 2011) and is becoming the most popular deep sequencing platform for diversity applications, including the Earth Microbiome Project (Gilbert et al., 2014). Currently, only a few cross-platform studies are available; these two methodologies have been compared in metagenomic studies (Luo et al., 2012), and other applications such identifying single nucleotide substitutions in whole genome sequences (Ratan et al., 2013). Regarding tag sequencing, comparisons have been performed in lake, soil or human samples (Claesson et al., 2010; Sinclair et al., 2015). The initial results showed that the taxonomic classification of reads from the first Illumina sequencers was worse than 454 due to their shorter length and higher error rates (Claesson et al., 2010). Nonetheless, the improvement in quality and length reads of later Illumina sequencers has shown promising results; Illumina performed in a similar manner than 454 with regards to estimates of alpha- and betadiversity except when estimating evenness in soil and lake samples (Sinclair et al., 2015). Here, a careful comparison of the performance of sequencing 16 and 18 S (for marine planktonic prokaryotes and small Picoeukaryotes, respectively) rRNA gene tags by using 454 and Illumina (pair-ended 2 × 250 bp) has been performed to determine and quantify marine picoplankton biodiversity, and the robustness of the results has been tested. The results show minor differences in the performance of both sequencing methodologies for rare taxa, but overall both methodologies provide a comparable view of marine planktonic biodiverstity. Moreover, we also show that certain taxa as well as the ratio between some phylogenetic groups may be good indicators of ecosystem health status. HTS may thus provide valuable information for the assessment of the environmental status in marine waters.

MATERIALS AND METHODS

Sample Collection and Basic Data

Surface waters were collected on 8th Aug 2013 in a 6 km inshore to offshore transect off the coast of Barcelona, NW Mediterranean. Five stations were sampled along the transect (**Figure 1**). Samples were sieved through a 200-µm mesh and transported to the laboratory within 2 h. Basic physical data was measured *in situ* with a conductivity, temperature and depth probe and surface salinity was analyzed with an AUTOSAL salinometer. The concentration of inorganic nutrients was determined spectrophotometrically by using an Alliance Evolution II autoanalyzer according to standard procedures (Grasshoff et al., 1983). Chlorophyll *a* (Chl *a*) concentration was measured from acetone extracts by fluorometry from the total fraction (<200 µm) and the fractions less than 20 and 3 µm. To collect microbial biomass, about 5 l of surface seawater was sequentially filtered through a 3- and a 0.2-µm pore-size polycarbonate filters (Poretics, GE Osmotics, Delft, Netherlands) using a peristaltic pump. The filters were stored in cryogenic vials containing 1.7 ml of lysis buffer (50 mM Tris-HCl pH 8.3, 40 mM EDTA pH 8.0 and 0.75 M sucrose) at −80°C until further processing.

DNA Extraction and Sequencing

The 0.2-µm filters were treated with lysozyme, proteinase K and sodium dodecyl sulfate, and the nucleic acids were extracted with phenol and concentrated in an Amicon 100 (Millipore), as described in Massana et al. (1997). DNA was quantified spectrophotometrically (Nanodrop, Thermo Scientific), and two subsamples from each extraction were sent for sequencing. Sequencing was performed by the Research and Testing

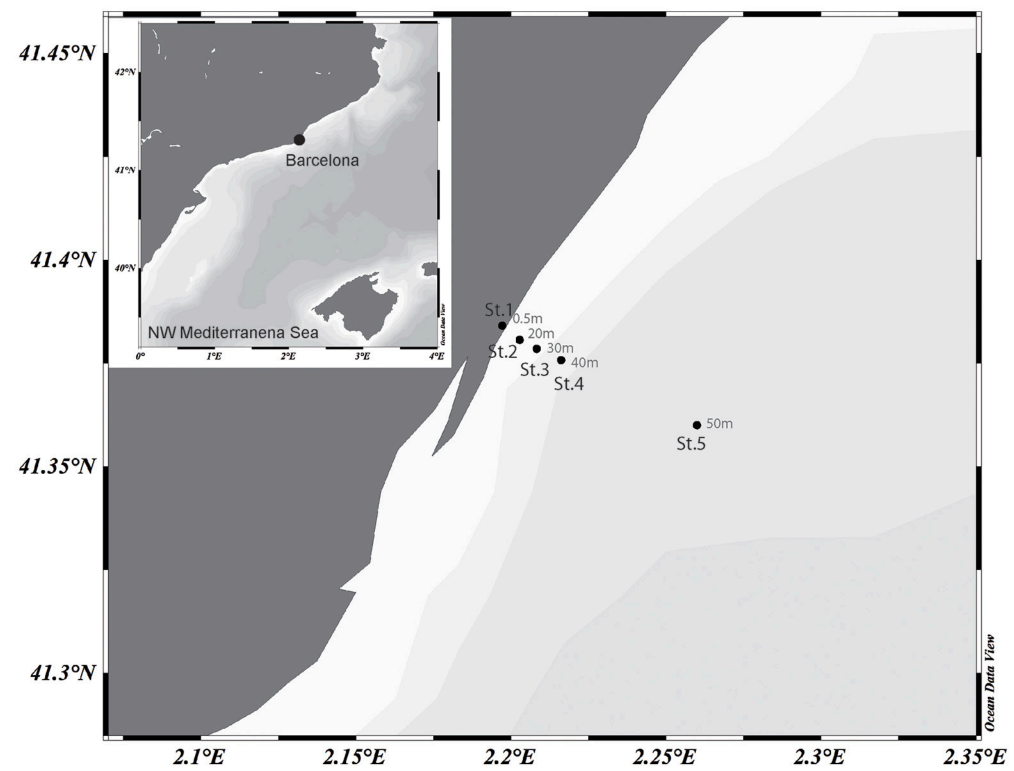

FIGURE 1 | Map showing the transect sampled off the coast of Barcelona in the NW Mediterranean Sea. Station location and depths (m) are indicated. The map was generated with the Ocean Data View Software (https://odv.awi.de).

Laboratory (Lubbock, TX, USA; http://www.researchandtesting.com/). Primers 341F (5′-CCTACGGGNGGCWGCAG-3′), and 805R (5′-GACTACHVGGGTATCTAATCC-3′) were used to amplify bacterial 16 S rRNA gene (Herlemann et al., 2011) and primers TAReukFWD1 5′-CCAGCASCYGCGGTAATTCC-3′ and TAReukREV3 5′-ACTTTCGTTCTTGATYRA-3′ were used to amplify the V4 region of the eukaryotic 18 S rRNA gene (Stoeck et al., 2010). Pyrosequencing was performed using the bTEFAP method by 454 GL FLX technology as described previously (Dowd et al., 2008). Illumina MiSeq 2 × 250 flow cells were used for Illumina sequencing following protocols described elsewhere (Cúcio et al., 2016). Approximately 30,000 raw sequences per sample were obtained.

Data Analyses

High-Performance computing analyses were run at the Marine Bioinformatics Service of the Institut de Ciències del Mar (ICM-CSIC) in Barcelona. Reads from the two sequencing methodologies underwent method-specific quality filtering before being pooled. Bacterial-454 data was filtered by quality using QIIME (Quantitative Insights Into Microbial Ecology, Caporaso et al., 2010) as described in Sánchez et al. (2013). Briefly, sequences from the 454 run were assigned a sample IDs using a mapping file and the barcode assigned to each sample. After sample IDs were assigned, bacterial sequences were removed from the subsequent analyses if they were shorter than 150 bp or longer than 500 bp, had an average quality score <25 calculated in sliding windows of 50 bp, contained more than two ambiguous characters or had an uncorrectable barcode. Eukaryotic-454 reads were quality checked and demultiplexed with QIIME following the same parameters described in Pernice et al. (2015). Shortly, sequences shorter than 150 bp or longer than 600 bp, with more than three mismatches in the primer, or having homopolymers longer than 8 bp were removed. Phred quality was analyzed in 50 bp running windows. Illumina sequences from bacteria and picoeukaryotes were quality filtered following a custom made pipeline (https://github.com/ramalok). Briefly, BayesHammer error correction of sequence reads was performed with SPAdes software (Nurk et al., 2013). Sequences were assembled with PEAR (http://pear.php.net/) and quality filtered in UPARSE (fastq_maxee value = 1). Clean bacterial-454 and bacterial-Illumina sequences were pooled and processed together; eukaryotic-454 and eukaryotic-Illumina sequences were also pooled together. Since 454 and Illumina sequences may have different length, bacterial sequences were truncated at equal depth (400 bp). However, for picoeukaryotes we did not truncate sequences, since large natural variability in the length of 18 S rRNA from different taxa occur. Sequences of both datasets were clustered into operational taxonomic units (OTUs) at 97% cutoff using the UPARSE algorithm implemented in USEARCH (Edgar, 2013). Both *de novo* chimera check and by comparison to reference database (SILVA) were done using the UCHIME algorithm (Edgar et al., 2011). Chimeric sequences and singleton OTUs (those represented by a single sequence) were removed. Taxonomic assignment of bacterial OTUs was performed using the BLAST classifier and the version 119 of the SILVA SSURef non-redundant database. OTUs assigned to chloroplasts were removed for subsequent analyses. For picoeukaryotes, OTUs were taxonomically classified by using BLAST against two reference databases: PR^2 (Guillou et al., 2013) and a marine microeukaryote database (MASS9013, Pernice et al., 2013). After taxonomic assignment, metazoan OTUs were removed. Sequence data has been submitted to the Genbank Sequence Read Archive under accession number SRP079955.

Statistical analyses were performed using the R statistical software (R Developement Core Team, 2015) and the packages *vegan*, *labdsv*, *venneuler*, *hmisc,* and *corrgram*. Alpha- and betadiversity analyses were performed using an OTU abundance table that was previously subsampled down to the minimum number of reads in order to avoid artifacts due to an uneven sequencing effort among samples. For alphadiversity analyses, we calculated the Chao1 index as a measure of richness and Shannon and Simpson indices as diversity metrics. Differences in microbial composition (betadiversity) were assessed using hierarchical clustering of Bray-Curtis dissimilarity matrices and the Unweighted Pair Group Method with Arithmetic Mean algorithm (UPGMA). To search for "indicator species" we used the IndVal (INDicator VALues; Dufrêne and Legendre, 1997) analysis, which identifies indicator species based on OTU fidelity and relative abundance. Only OTUs with significant p-values (< 0.05), and >0.3 IndVal values were considered. To assess links between diversity and environmental data we performed linear regressions and pairwise correlations (Pearson's correlation coefficient). The results were thresholded at $p < 0.05$. Analysis of variance was run to test for differences among diversity data and categories (sequencing method, station) with Tukey-Kramer *post hoc* comparisons at the 5% significance.

RESULTS

Five stations were sampled in an inshore-to-offshore transect off the coast of Barcelona. Station 1 was located closest to the shore. The following stations were sampled in increasing depth and distance to the shore (**Figure 1**). Basic physicochemical data is shown in **Table 1**. The sampled area is expected to suffer impacts from human activities due to a large urban development, and putatively receiving pollutants from urban and industrial activities (domestic waste, organic and inorganic nutrient enrichment). A decreasing nutrient concentration was observed as distance to shore increased. Despite this variability can in part be associated to natural processes, it can also reflect the degree of human impact (i.e., nutrient enrichment). Concentration of all nutrients measured showed the lowest values in Station 5 (offshore) and higher values closer to the shore.

Influence of the Sequencing Platform on Microbial Diversity

Sequencing of all bacterial and most picoeukaryotic samples was successful yet two picoeukaryotic replicates (1a, 454, and Illumina) resulted in a low number of reads and were discarded from further analyses. The bacterial dataset resulted in 277,212 high quality reads that clustered in a total of 658 OTUs at 97% similarity. From those only 34.7% of OTUs were shared

TABLE 1 | Values of physicochemical variables measured along the inshore-to-offshore transect.

Station	Temperature (°C)	Salinity	Chl *a* total [μg l^{-1}]	Chl *a* < 3 [μg l^{-1}]	Chl *a* 3–20 μm [μg l^{-1}]	Chl *a* > 20 [μg l^{-1}]	PO_4^{3-} [μM]	NH_4^+ [μM]	NO_2^- [μM]	NO_3^- [μM]	SiO_4^{4-} [μM]
1	nd	37.87	1.14	0.23	0.31	0.55	0.35	3.95	0.98	3.91	4.44
2	25.87	37.90	1.94	0.91	1.03	1.94	0.13	1.35	0.36	1.03	1.12
3	25.85	37.91	2.09	1.18	0.91	2.09	0.12	0.94	0.14	0.47	1.10
4	nd	37.92	1.08	0.54	0.48	1.02	0.09	1.60	0.20	0.49	0.83
5	25.65	37.04	0.22	0.09	0.13	0.22	0.05	0.78	0.05	0.19	0.65

between samples sequenced by either 454 or Illumina (**Figure 2**). However, the unique OTUs in each methodology correspond to rare members; the proportion of shared OTUs (350 out of 658) represented 99.4% of the reads. We found a good correlation between the relative abundance of each OTU sequenced by both methodologies ($R = 0.87$, $p < 0.001$). Likewise, when grouping OTUs into the main bacterial taxa, a good agreement between contributions obtained by 454 or Illumina was found ($R = 0.81$, $p < 0.001$). In both cases, most bacterial sequences were related to the phyla Proteobacteria (average of all bacterial dataset, 72%), Bacteroidetes (20%), and Cyanobacteria (5%). Within the Proteobacteria, the most prevalent classes were the Alpha-(50%) and the Gammaproteobacteria (20%), whereas the Beta-, Delta-, and Epsilon- were present at low relative abundances (grouped as "Other Proteobacteria," **Figure 3**). Within the Alphaproteobacteria, the OTUs showing higher relative abundances were affiliated to the Rhodobacterales, Rhodospirillales, Rickettsiales, and the SAR11 clade. The Bacteroidetes were largely represented by members of the Flavobacteriia. The Actinobacteria represented on average 1% of the total reads. Several other groups such as the Acidobacteria, Firmicutes, Gracilibacteria, Parcubacteria, Planctomycetes, and the Verrucomicrobia were also detected but at low read abundances (<1%) and were grouped as "Other Bacteria" for plotting purposes. Analysis of the variance resulted in no significant differences in the contribution of the major taxa retrieved by each sequencing methodology.

A similar pattern was observed for picoeukaryotes (0.2–3 μm size fraction). The 556,143 clean reads were clustered into an OTU table at 97% similarity that contained 768 OTUs; from those only 37.1% were shared between the two methodologies, but these represented the vast majority of reads (96.4%; **Figure 2**). OTUs recovered with only one of the sequencing methodologies represented very rare members. In fact, as for bacteria, we found very good correlations when comparing the relative abundance of the different taxa both at the OTU level or clustering them at the taxonomic group level ($R = 0.84$, $p < 0.001$, and $R = 0.91$, $p < 0.001$, respectively; see **Figure 3**). The picoeukaryotic OTUs were classified into 70 class-level groups. The taxonomic affiliation was dominated by four groups that accounted on average for >55% of the total number of reads within the picoeukaryotic dataset: Mamiellophyceae (19% of the reads, dominated by *Micromonas* OTUs [97% of Mamiellophyceae]), Dinophyceae (17%), MALV-II (10%), and Cryptophyceae (10%). Other less abundant groups included MALV-I, Chlorarachnida, Picozoa, Prasinophyceae, Dictyochophytes, Chlorodendrophyceae, MAST-3, and Pelagophytes. The remaining 58 taxonomic groups presented very low relative abundances (<1.1%) and were grouped as "Other Eukaryotes." No statistically significant differences in the relative abundance retrieved by 454 or Illumina for the difference groups were found.

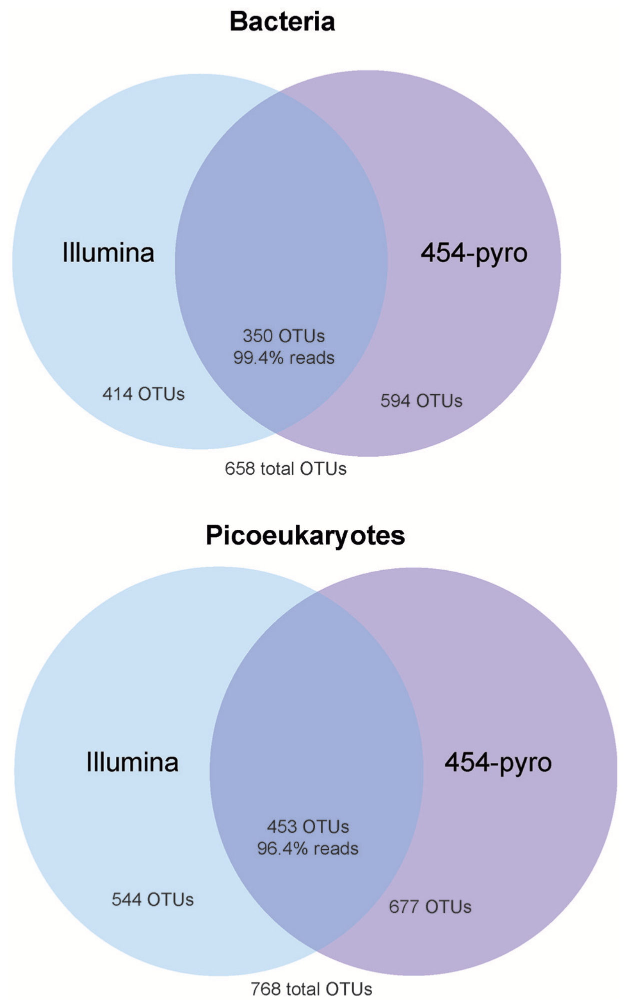

FIGURE 2 | Venn diagrams showing the extent of overlapping and platform-specific OTUs between the 454 and Illumina datasets applied to the bacterial (top) and picoeukaryotic (bottom) analyses.

In order to further explore whether the sequencing methodology had an influence on the bacterial and

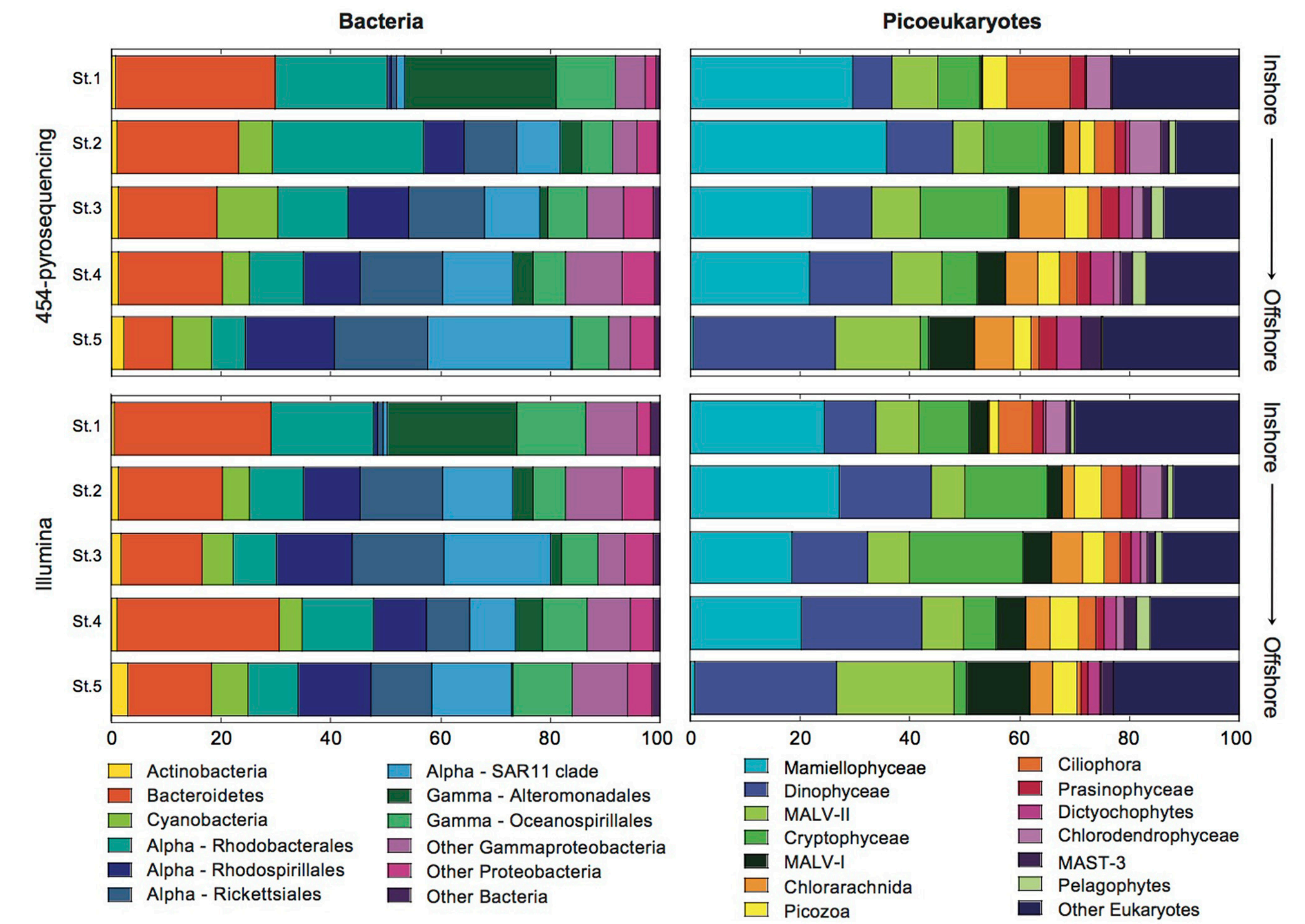

FIGURE 3 | Bar graphs showing the relative contributions of the major bacterial and picoeukaryotic taxa to community structure in the coastal (Station 1) to offshore (Station 5) transect as retrieved using 454 and Illumina sequencing.

picoeukaryotic diversity, we calculated various widely used indices of alphadiversity: the Chao 1 index for richness, and the Shannon and Simpson indices for diversity estimation (Hill, 1973; Magurran, 1988; Chao and Lee, 1992; **Figure 4**). Analysis of variance showed no significant differences between sequencing platforms for any of the indices tested, neither for Bacteria nor for Picoeukaryotes ($P > 0.05$). Additionally, to infer the variation of the microbial assemblages along the gradients, that is, beta diversity, the Bray–Curtis dissimilarity index was used on community composition. Dissimilarity matrices were constructed based on the relative abundance of each OTU. The distance between samples and replicates was visualized using hierarchical clustering. The results show that, in general, replication was good within each sequencing platform, but replicates sequenced using the same methodology were more similar among each other, which indicates that the sequencing chemistry has a certain influence on the community composition observed (**Figure 5**). For Bacteria, the samples grouped according to station regardless of the sequencing platform, except for Stations 3 and 4, which grouped by method, indicating that the platform introduces errors and artifacts to a certain extent at the OTU level. A similar trend was observed for the picoeukaryotic dataset, in which samples grouped by station, and in general were more similar among replicates subjected to the same methodology. Yet, in one case, the replicate obtained by Illumina (Illu−4a) was fairly different to the rest of the replicates from the same station. The number of OTUs in Illu-4a sample was much lower than in the other three replicates of Station 4 (one from Illumina and two from 454), indicating some biases in amplification or sequencing of this specific sample.

Bacterial and Picoeukaryotic Plankton Diversity along a Inshore-to-Offshore Gradient

In order to obtain direct descriptors of the bacterial and picoeukaryotic diversity of plankton assemblages, we compared the diversity retrieved along the inshore-to-offshore gradient (**Figure 1**, **Table 1**). We observed significant differences in alphadiversity between stations (**Figure 6**). In particular, significant differences for Chao1 and Simpson indices were found for the bacterial dataset. The Chao1 showed a clear

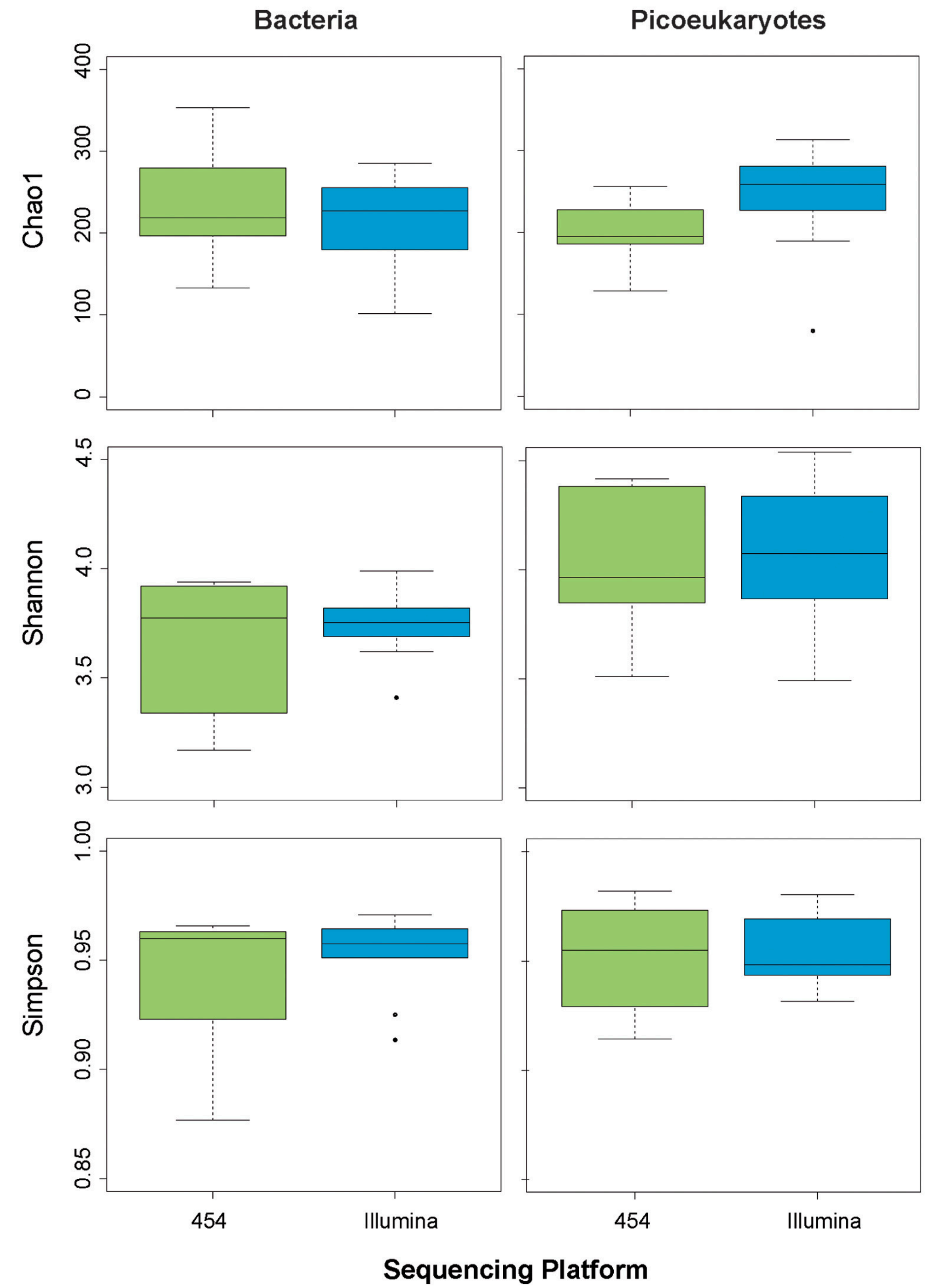

FIGURE 4 | Box plots showing various estimates of alphadiversity (Chao1, Simpson, Shannon) depending on sequencing methodology for bacteria (left panels) and picoeukaryotes (right panels).

inshore-to-offshore decrease, whereas the Simpson index showed higher values in the transition zone from the coastal to the offshore station (**Figure 6**). The Shannon index showed a similar trend to the Simpson index but the differences detected were not significant. Interestingly, eukaryotic picoplankton showed a different trend. Whereas the values of Chao1 were quite constant along the gradient, the Shannon and Simpson diversity indices increased from coast to offshore. In fact, statistical analyses (ANOVA) confirmed significant differences, particularly between stations 1 and 5.

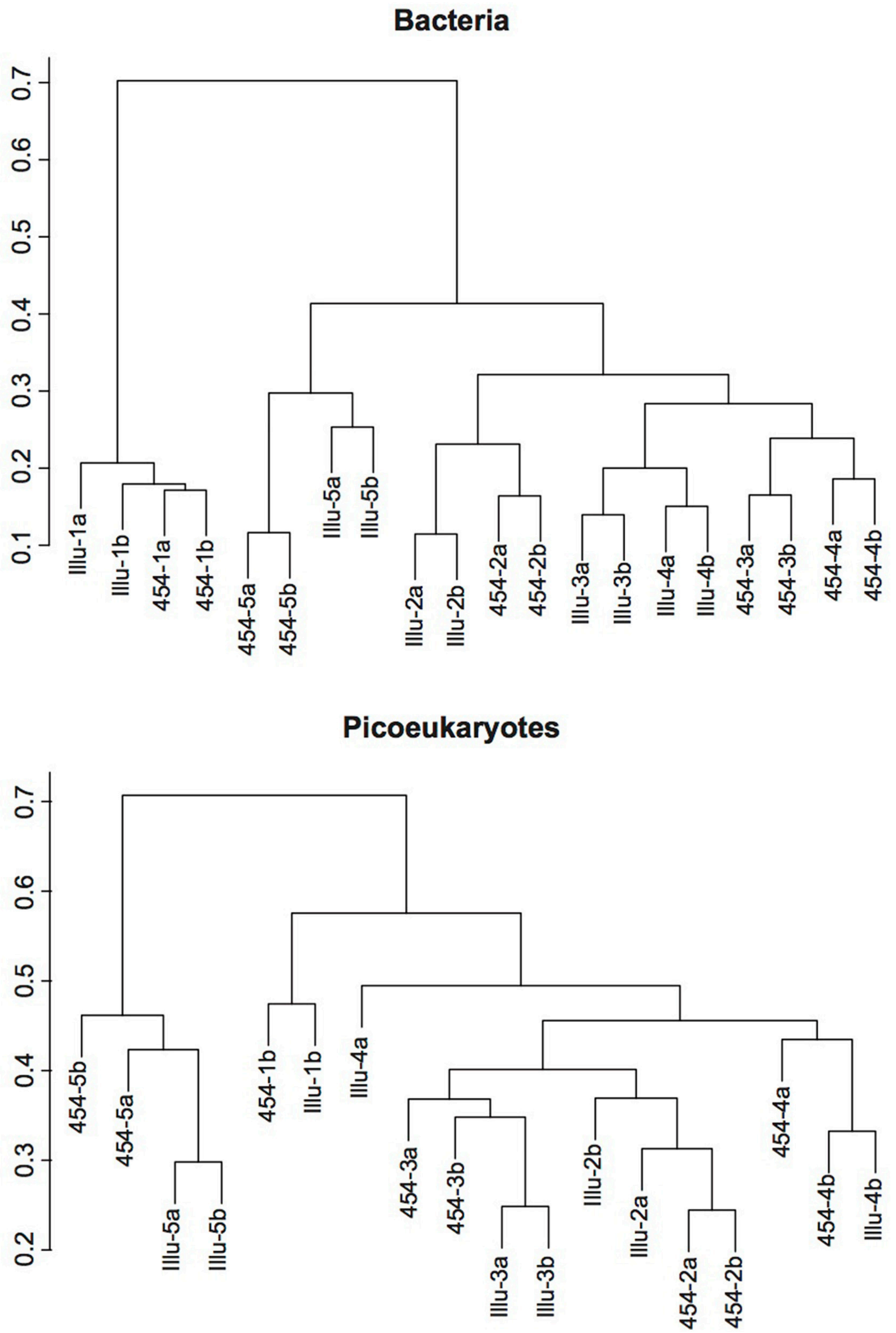

FIGURE 5 | Hierarchical clustering dendograms representing Bray-Curtis dissimilarities for the bacterial (top) and picoeukaryotic (bottom) samples. The samples are coded with the platform used (Illu for Illumina, and 454 for 454-pyrosequencing), the station number (from 1, the coastal station, to 5, the offshore one), and the replicate (a, b).

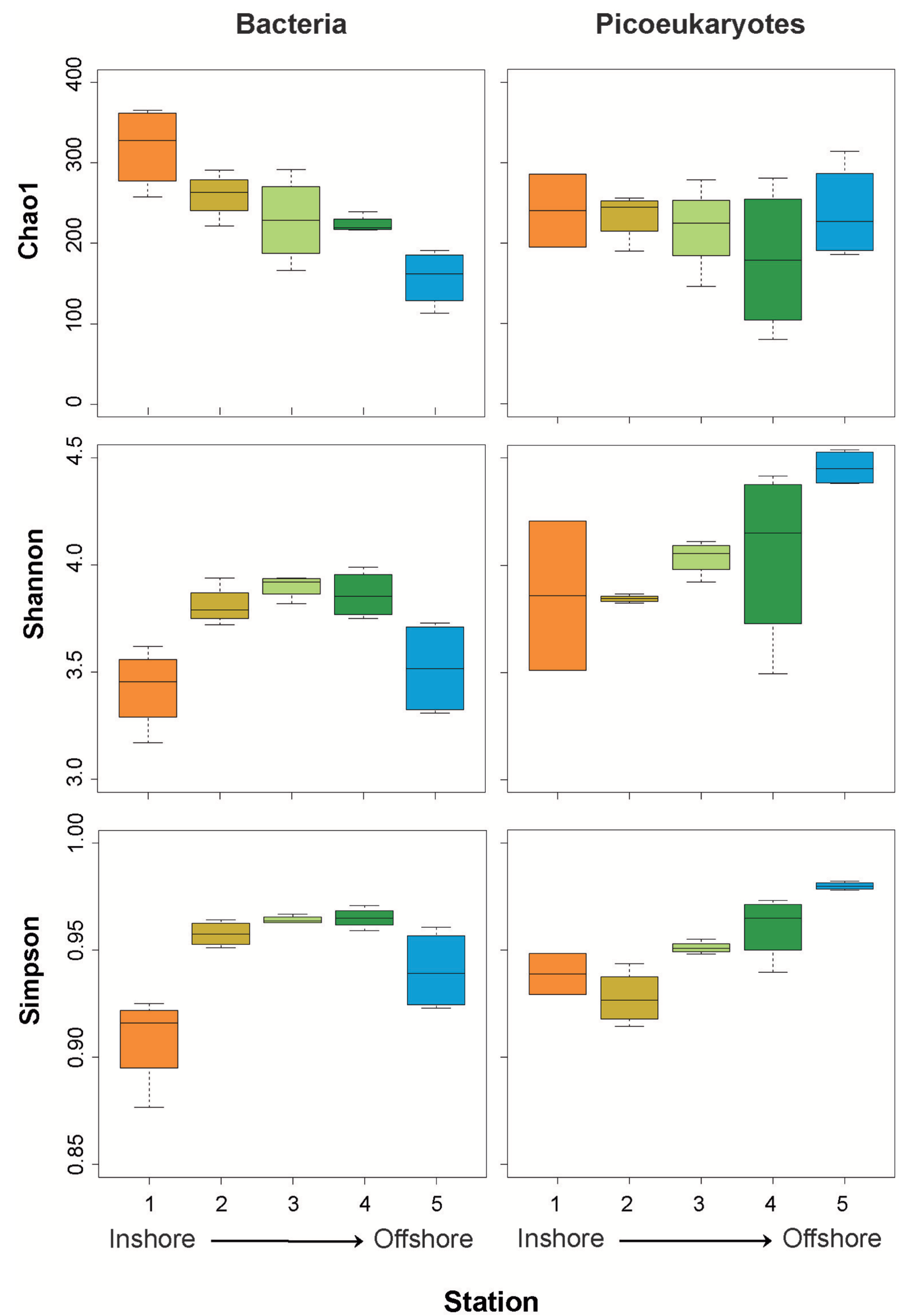

FIGURE 6 | Box plots showing estimates of alphadiversity (Chao1, Simpson, and Shannon indices) from the coastal (Station 1) to the offshore station (Station 5) for bacteria (left panels) and picoeukaryotes (right panels).

In addition, clear inshore-to-offshore changes in community composition were found both for bacteria and eukaryotic picoplankton. The larger differences were detected between Station 1 (coastal) and Station 5 (offshore), whereas a transition in community composition was observed at intermediate stations (**Figure 3**). In the case of Bacteria, some phylogenetic groups (Phylum, Class, and Order levels) showed a clear increase in their abundance from coast to offshore. These include the phylum Actinobacteria and the orders Rhodospirillales, Rickettsiales, and SAR11 within the class Alphaproteobacteria. An opposite trend was observed for the phylum Bacteroidetes, order Rhodobacterales (Alphaproteobacteria), and orders Alteromonadales and Oceanospirillales of the Gammaproteobacteria. Phylum Cyanobacteria were small contributors to community composition in the coastal station and peaked at Station 3 coinciding with the highest value of Chl *a*. The greater differences were observed for the order Alteromonadales which represented >25% of the reads in the coastal station and decreased to almost nil in the offshore station. Conversely, the SAR11 clade increased from 1 to >20% of the reads along the transect. Analyses of variance confirmed significant differences between stations for all the above-mentioned groups (details now shown).

The picoeukaryotic community also changed along the gradient being likewise Station 1 the most different from Station 5. The lineage Mamiellophyceae showed similar high relative abundance (>20%) in all stations except in Station 5, where they were virtually absent. The relative abundance of Cryptophyceae increased from Station 1 to Station 3 and then decreased toward offshore stations. Dinophyceae, Dictyochophytes, marine alveolates (MALV-II and MALV-I), and Stramenopiles showed increasing contributions along the transect. Contrarily, Ciliophora were important contributors only in the coastal station. Other groups presented quite constant contributions in all stations (Picozoa, Prasinophyceae, Dictyochophytes; **Figure 3**).

Potential Indicators of Environmental Status

Potential "indicator species" were explored by calculating the indicator value (IndVal; Dufrêne and Legendre, 1997; Podani and Csányi, 2010) which identifies indicator species based on species (or OTU) fidelity and relative abundance, both for bacterioplankton and eukaryotic picoplankton. The IndVal of a species is a popular measure to express species importance in community ecology. Its potential to measure species explanatory power and to reflect environmental quality has been explored in biodiversity surveys (Gevrey et al., 2010; Keith et al., 2012; Lumbreras et al., 2016). We classified the stations into three categories, i.e., coastal (Station 1), transition (Stations 2, 3, and 4), and offshore (Station 5) and searched for indicator OTUs. We found 114 bacterial OTUs with significant IndVal values, potentially useful as indicator species. However, we considered only those OTUs showing (i) IndVal values >0.3, as this is the value that has been proposed to be a good threshold for habitat specialization (Dufrêne and Legendre, 1997), and (ii) overall relative abundance >1% since the potential as indicator species of rare OTUs is questionable taking into account the differences found between sequencing methods for the rare OTUs and the known biases of the PCR-based methodologies (Polz and Cavanaugh, 1998; Acinas et al., 2005). After selection, the list was reduced to 23 bacterial OTUs. We found OTUs with explanatory power for all three categories. The OTU with higher IndVal value was affiliated to a species of Gammaproteobacteria (*Marinobacterium*) and was indicative of coastal waters. On the contrary, alphaproteobacterial members of the SAR11 clade were explanatory for offshore waters and mainly Bacteroidetes for the transition zone. Within picoeukaryotes, a total of 164 OTUs with significant values were found but after filtering the table using the same criteria only 13 OTUs were retained. Most of them were explanatory for Station 5 in offshore waters. However, the indicator OTU presenting a higher contribution, OTU1, was classified as *Micromonas pusilla*, and was indicator for coastal waters in agreement with previous reports that have shown the preference of *Micromonas* species for coastal waters (Not et al., 2005, 2008). Overall, IndVal values for picoeukaryotic OTUs were lower than for bacterial OTUs. In both cases, the highest values were associated to rare species (details not shown) that were discarded based on abundance data. The selected IndVal scores and associated OTUs are listed in **Table 2**.

In addition to exploring potential "indicator species," we explored the microbial profiles as possible descriptors of environmental status. That is, analyzing the relative abundance of the most abundant phylogenetic groups in each sample in relation to the degree of impact. The transect analyzed off the coast of Barcelona reflects a decreasing gradient of human impact from inshore (Station 1) to offshore (Station 5) which is somewhat reflected in the concentration of inorganic nutrients (see **Table 1**). The analysis of changes in community composition along the gradient together with the OTUs showing highest IndVal scores suggested the exploration of the ratios between taxa as potential indices of ecosystem health status. Interestingly, we found strong positive and negative correlations between the relative abundance of different bacterial groups as well as the ratio between taxa and the concentration of nutrients. The strongest correlation detected was a positive correlation between the relative abundance of Alteromonadales and all nutrients measured (phosphate, nitrite, nitrate, ammonium, silicate, $R > 0.96$, $p < 0.0001$). Likewise, the ratios Alphaproteobacteria/Gammaproteobacteria, *Alteromonas*/SAR11, and *Alteromonas* + Oceanospirillales/SAR11 were strongly correlated to nutrient concentration ($R > 0.90$, $p < 0.0001$). For picoeukaryotes, we found significant correlations between the relative abundance of certain taxa and the nutrient load, yet these correlations were in general weaker than for bacteria. The strongest positive correlations were found for Ciliophora and all nutrients ($R = 0.85$–0.88, $p < 0.0001$). Significant negative correlation between Chlorarachnida and nitrite ($R = 0.79$) and nitrate ($R = 0.75$) as well as between Dinophyceae and phosphate ($R = 0.71$) were also observed.

DISCUSSION

Do Different Sequencing Methodologies Provide Comparable Views of Microbial Biodiversity in Marine Ecosystems?

Up to date, several studies have investigated the potential biases on the estimations of richness and evenness in microbial communities associated with the primer selection and the PCR

TABLE 2 | Potential indicator OTUs identified with IndVal.

	OTU	Zone	IndVal	*p*-value	Abundance (%)	Accession number	Taxonomy	Similarity (%)
Bacteria	OTU10	C	0.90	0.001	1.9	AM747353	Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; uncultured	100
	OTU16	C	0.90	0.001	1.1	KF799685	Proteobacteria; Gammaproteobacteria; Oceanospirillales; Oceanospirillaceae; *Marinobacterium*	100
	OTU1	C	0.88	0.001	7.2	HM591452	Proteobacteria; Gammaproteobacteria; Alteromonadales; Alteromonadaceae; *Glaciecola*	100
	OTU4	C	0.84	0.002	2.7	KC425546	Bacteroidetes; Flavobacteriia; Flavobacteriales; Cryomorphaceae; *Owenweeksia*	100
	OTU8	C	0.77	0.001	1.7	JN625680	Proteobacteria; Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; *Nereida*	100
	OTU20	C	0.68	0.001	1.4	GQ347928	Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; *Formosa*	100
	OTU19	C	0.58	0.001	1.2	DQ009134	Proteobacteria; Gammaproteobacteria; Cellvibrionales; Halieaceae; OM60(NOR5)_clade	100
	OTU32	T	0.67	0.001	1.7	EU805333	Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; NS5_marine_group	99.7
	OTU40	T	0.53	0.008	1.5	KC000574	Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; NS4_marine_group	100
	OTU14	T	0.51	0.013	1.5	HM117554	Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; NS5_marine_group	99.7
	OTU12	T	0.50	0.005	2.3	JX206780	Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; NS5_marine_group	99.7
	OTU160	T	0.49	0.035	1.2	KC000216	Proteobacteria; Gammaproteobacteria; Cellvibrionales; Halieaceae; OM60(NOR5)_clade	99.4
	OTU24	T	0.47	0.027	1.1	EU799445	Bacteroidetes; Flavobacteriia; Flavobacteriales; Flavobacteriaceae; NS5_marine_group	99.7
	OTU15	T	0.46	0.018	1.4	EU799321	Bacteroidetes; Cytophagia; Order_III; Unknown_Family; *Balneola*	100
	OTU21	O	0.74	0.001	5.6	KC002232	Proteobacteria; Alphaproteobacteria; SAR11_clade; Surface_1; unidentified_marine_bacterioplankton	100
	OTU26	O	0.65	0.001	2.4	KC002796	Proteobacteria; Alphaproteobacteria; SAR11_clade; Surface_2	100
	OTU5	O	0.63	0.004	1.9	FJ745058	Proteobacteria; Alphaproteobacteria; Rickettsiales; SAR116_clade	100
	OTU6	O	0.60	0.001	8.4	AACY020487638	Proteobacteria; Alphaproteobacteria; Rhodospirillales; Rhodospirillaceae; AEGEAN-169_marine_group	99.7
	OTU17	O	0.58	0.001	1.6	EU805027	Proteobacteria; Alphaproteobacteria; Rickettsiales; S25-593	100
	OTU29	O	0.57	0.017	1.1	FJ745253	Proteobacteria; Gammaproteobacteria; Oceanospirillales; Litoricolaceae; *Litoricola*	99.4
	OTU11	O	0.56	0.008	3.3	EF076075	Proteobacteria; Alphaproteobacteria; Rickettsiales; SAR116_clade	100
	OTU3	O	0.55	0.006	5.1	KC000519	Cyanobacteria; Cyanobacteria; SubsectionI; FamilyI; *Synechococcus*	100
	OTU13	O	0.46	0.001	3.1	AACY023421318	Proteobacteria; Gammaproteobacteria; Oceanospirillales; SAR86_clade	100
Picoeukaryotes	OTU12	C	0.68	0.012	1.0	AY425301	Archaeplastida; Chlorophyta; Chlorodendrophyceae; Chlorodendrales; Chlorodendrales sp.	99.0
	OTU1	C	0.55	0.027	13.6	KT860903	Archaeplastida; Chlorophyta; Prasinophyceae; Mamiellales; Mamiellaceae; *Micromonas pusilla*	100
	OTU24	T	0.95	0.001	1.0	AF076172	Rhizaria; Cercozoa; Filosa-Chlorarachnea; Chlorarachnida; Norrisiella; *Norisiella sphaerica*	86.0
	OTU5	T	0.69	0.005	1.6	KT860555	Hacrobia; Cryptophyta; Cryptophyceae; Pyrenomonadales; Geminigeraceae; *Teleaulax* sp.	99.0
	OTU2	T	0.58	0.014	6.9	EF527054	Hacrobia; Cryptophyta; Cryptophyceae; Cryptophyceae; Cryptomonadales; Cryptomonadales sp.	100
	OTU8	O	0.79	0.004	1.1	KF130174	Hacrobia; Centroheliozoa; Centroheliozoa; Pterocystida; Pterocystida; Pterocystida sp.	100
	OTU6	O	0.67	0.002	2.3	KJ763319	Rhizaria; Cercozoa; Filosa-Chlorarachnea; Chlorarachnida; Partenskyella; *Partenskyella glossopodia*	99.7
	OTU3	O	0.63	0.018	2.2	KJ763079	Alveolata; Dinophyta; Syndiniales; Dino-Group-I; Dino-Group-I-Clade-1; Dino-Group-I-Clade-1 sp.	100
	OTU17	O	0.62	0.004	2.0	KJ763233	Alveolata; Dinophyta; Dinophyceae; Dinophyceae; Dinophyceae; Dinophyceae sp	100
	OTU11	O	0.58	0.01	1.6	KJ760683	Alveolata; Dinophyta; Syndiniales; Dino-Group-II; Dino-Group-II-Clade-10-and-11; Dino-Group-II-Clade-10-and-11 sp.	100
	OTU13	O	0.58	0.021	2.6	KJ763398	Alveolata; Dinophyta; Dinophyceae; Dinophyceae; Dinophyceae; Dinophyceae sp.	99,0
	OTU34	O	0.57	0.004	1.2	EU793918	Alveolata; Dinophyta; Syndiniales; Dino-Group-I; Dino-Group-I-Clade-1; Dino-Group-I-Clade-1 sp.	100
	OTU156	O	0.56	0.018	3.8	KJ763064	Alveolata; Dinophyta; Dinophyceae; Dinophyceae; Dinophyceae; Dinophyceae sp.	99.5

For each OTU, we indicate the zone for which was indicator (C, coastal; T, transition; O, offshore), the Indicator Value (IndVal), the statistical significance of the association (p-value), the average relative contribution to community structure of the OTU in the dataset (abundance), the GenBank accession number, the taxonomy and the similarity to the reference database used (see Section Material and Methods).

step in amplicon-based studies (Acinas et al., 2005; Hong et al., 2009; Engelbrektson et al., 2010; Parada et al., 2015). However, since cross-platforms studies are rare, currently it is unclear whether the inherent differences in chemistry and sequencing protocols will affect the quality of the sequences and the estimates of genetic diversity and community structure. Furthermore, despite variability is known to be introduced during sample manipulation, PCR amplification and sequencing, the numerous studies on microbial diversity using HTS lack analysis of replicates (Prosser, 2010). For these reasons, we compared the two most frequently used HTS platforms, the Roche 454 FLX Titanium, and the Illumina MiSeq, on a set of DNA samples obtained from an inshore-to-offshore transect in the coast of Barcelona. Additionally, we explored the reproducibility of the results by sequencing replicates. Overall, the platforms provided a comparable view of the marine picoplankton communities but some differences were found when comparing the datasets at the OTU level.

Different HTS platforms produce millions of short sequence reads, which vary in length. It is known that sequence length can impact diversity estimates (Claesson et al., 2010). Nowadays, pair-end Illumina can produce up to 300 bp nucleotide reads, and thus is feasible to do a careful compassion with 454 using the same primer set, providing the same amplicon length, and thus distinguish the performance of both methodologies based only in potential differences in the chemistry of the sequencing. Here, we found that the sequencing methodology does not significantly influence estimates of alphadiversity. No significant differences in Chao1, Shannon, and Simpson indices were found between platforms. A recent study comparing the Illumina and 454 platforms to study bacterial diversity via 16 S rRNA gene amplicons in sediments and soda lakes also found that both methodologies performed in a similar manner and that the general trends in alphadiversity were conserved with the exception of evenness estimates where correspondence between methods was low (Sinclair et al., 2015). It is known that the OTU clustering method can influence the estimates of diversity (Edgar, 2013; Flynn et al., 2015; Sinclair et al., 2015). We used the UPARSE algorithm, which offers an improved accuracy compared to other methods, resulting in fewer OTUs likely closer to the expected number of species in a community (Edgar et al., 2011). Using this methodology may have reduced the influence of sequencing and amplification artifacts and resulted in comparable estimates of diversity by the two sequencing methodologies.

We did observe some differences for betadiversity, that is the variation of the microbial assemblages along the transect, despite the trends identified were in general similar for both methodologies. Replication was good within each sequencing platform but in general replicates were more similar among each other depending on methodology, revealing thus a certain influence of its chemistry. We found that the bacterial communities in Stations 3 and 4 were more similar depending on the method indicating that the platform introduces biases. Oceanographic conditions were quite similar between these two stations (**Table 1**), and therefore microbial communities could be expected to be fairly similar. Sampling artifacts associated with random sampling (Zhou et al., 2008), PCR biases (Polz and Cavanaugh, 1998; Acinas et al., 2005) or errors directly related to the performance of the technology *per se* (Berry et al., 2011; Schirmer et al., 2015) can occur at any time, but when comparing samples, the impact of these artifacts will depend on the similarity among those samples. In this case, it is feasible to assume that the potential artifacts associated to the methodology overwhelmed the natural differences between the communities in these closer stations. For picoeukaryotes, in general samples grouped by station as expected, indicating that the sequencing biases, if any, were minor. However, there is one replicate from Station 4 that differs substantially from the other replicates. Problems during PCR amplification or degradation of the DNA could explain this difference.

Venn diagrams showed that less than half of the total OTUs were equally retrieved by both methodologies. However, the non-shared OTUs correspond to very rare contributors of these microbial communities. The concept of the rare biosphere has attracted a lot of attention in the last years (Pedrós-Alió, 2012; see reviews by Lynch and Neufeld, 2015). Microbial communities are dominated by a small number of species that account for most of the biomass and a large number of species that are represented by only a few individuals (rare members). The development of HTS has allowed accessing at least some of these rare microbial species. However, it is known that some of the rare OTUs retrieved in microbial diversity surveys correspond to sequencing errors (Kunin et al., 2010). We discarded the singletons (OTUs represented by a single sequence in the whole dataset) to avoid potential artifacts in diversity estimates. Nevertheless, still over half of the OTUs were only retrieved by one methodology. Part of it can be explained because rare OTUs may or may not appear in a dataset only by random chance but we cannot discard that part of this diversity is due to sequencing errors. For that reason, for the purpose of finding indicator species, we decided to focus only on those OTUs that represented >1% of the total relative abundance. Regardless of the differences in the rare OTUs, the two sequencing technologies revealed very similar profiles when grouping OTUs at the class and family levels (**Figure 3**). Relative taxa abundances were consistent across technologies and thus, the view of the community composition was fairly comparable. The results show that, due to the improvement in the length of Illumina sequence reads, Illumina tags offer similar classification efficiencies than 454 tags at a much lower cost (Glenn, 2011), being therefore a cost efficient approach for biodiversity monitoring.

Does Plankton Diversity Have Informative Potential for Environmental Status Assessment?

Diversity and trophic state are two quality descriptors for evaluating ecosystem function in the MSFD. Despite the main goal of this work was to compare HTS methodologies for biodiversity monitoring, we further explored whether picoplankton biodiversity can be used as an alternative indicator of environmental status. The Mediterranean Sea is a valuable paradigm to assess anthropic pressure, because of the contrasting nature of its offshore and coastal areas. The offshore waters of the Mediterranean Sea are among the most oligotrophic areas of the world. In these waters, nutrient availability is low and inorganic phosphorus concentrations limit primary production. On the contrary, coastal areas are nutrient rich, as they receive river discharges, runoff from populated areas, and submarine groundwater, but they are also influenced by offshore oceanographic conditions. The coastal marine zone is therefore a transitional area characterized by strong physical, chemical, and biological gradients that extend from land to sea. Here, biological production is closely coupled to processes that deliver nutrients to surface waters. Anthropogenic forcing clearly influences the absolute availability of these nutrients and their stoichiometry, both of which impact phytoplankton productivity and species composition (Camp et al., 2015). The studied transect is expected to have a decreasing degree of anthropogenic pressure as the distance from the coast increases (from Station 1 to 5). Concentration of inorganic nutrients, as indication of eutrophication, showed indeed a decreasing concentration. We determined common alphadiversity indices as possible descriptors of the environmental status since pressures can lead to changes in microbial composition (Torsvik et al., 2002; Smith and Schindler, 2009) and those could reflect variations in biodiversity. For bacteria, Shannon and Simpson indices showed a similar trend with higher values at intermediate stations of the transect. The observed trend could be explained by the "intermediate disturbance hypothesis" (Connell, 1978), which suggests that intermediate intensity of disturbance maximizes diversity, and therefore systems with low and high disturbance, such Stations 5 and 1 in terms of nutrient load, can harbor similar levels of diversity. In any case, as previously observed in other systems (Garrido et al., 2014) these indices do not seem promising as indicators to asses environmental status. Contrarily, a clear decrease in richness was observed from coast to offshore. A sharp decrease of richness from coastal to offshore locations in the NW Mediterranean has been previously documented (Pommier et al., 2010). On the other hand, an increase in Shannon and Simpson indices was observed along the transect for picoeukaryotes, indicating a higher diversity in more oligotrophic stations (Cheung et al., 2010). Furthermore, the most abundant OTU in all stations but Station 5, *Micromonas*, is known to be more common in coastal areas than open ocean (Not et al., 2005), possibly related to higher nutrient load in coastal waters. The results found here suggest that it may be worth exploring the links between bacterial and picoeukaryotic diversity and environmental status on coastal waters over time and space covering a wide range of impacts.

Traditionally, several species of plants and animals have been and still are being used as indicator species for different types of pollution in monitoring programs (Borja et al., 2000, 2008; Ferrat et al., 2003; Montefalcone, 2009; Marbà et al., 2013). Likewise, plankton indicators have been proposed for diagnoses of ecosystem state (Beaugrand, 2005). Most studies have focused on species of zooplankton (i.e., *Calanus finmarchicus*) or some phytoplankton bloom-forming species. For example, *Phaeocystis* sp. produces spring blooms in the North Sea which magnitude might indicate an excess of available N or P in relation to dissolved silica and thus, is considered and indicator for eutrophication (Tett et al., 2007). However, several flaws in the usefulness of using large phytoplankton to reflect significant pressure-impact relationships have been identified (Cloern and Jassby, 2008, 2010; Camp et al., 2015). Bacterial and eukaryotic picoplankton constitute the smallest but most abundant organisms of plankton and are key players in ecosystem functioning. Since disturbances can affect community structure and ecosystem functioning, the smallest members of marine plankton may be crucial in understanding the magnitude of these disturbances particularly because of their fast response to environmental change. In fact, microorganisms have been already proposed as indicators of marine environmental quality, and not only the presence of pathogens such as *E. coli*, commonly used as indicator of fecal contamination, but in relation to biodiversity and ecosystem functioning (Caruso et al., 2015). Here we tested the Indicator Species Value from Dufrêne and Legendre (1997) in the different sampled stations. The IndVal identifies indicator species based on OTU fidelity and relative abundance. Different bacterial and picoeukaryotic OTUs showed high scores for Stations 1 and 5, as well as for intermediate stations and could represent potential "indicator species." Alternatively to "indicator species," we explored the potential of using the abundance of certain taxa and the ratio between different groups of microorganisms as an alternative indicator of environmental status. These indices may also offer ecological information (i.e., species relative composition). In fact, this approach has been explored in other ecosystems; for example in reclaimed waters, the ratio between the Bacteroidetes, Gammaproteobacteria, and *Nitrospira*/Betaproteobacteria (BGN:β) seems a possible alternative indicator of water quality (Garrido et al., 2014). We tested the correlation of different taxa and the degree of eutrophication (i.e., nutrient concentration) and found significant correlations between certain picoeukaryotic taxa e.g., Ciliophora, and nutrient load; this taxa has been found previously in high abundances in eutrophic waters (Romari and Vaulot, 2004). Yet, the strongest correlations were with the ratio of Alphaproteobacteria/Gammaproteobacteria, *Alteromonas*/SAR11, and *Alteromonas* + Oceanospirillales/SAR11. Whether these "indicator species" and indices can be used as robust alternative indicators of environmental status remains to be explored in different locations subjected to contrasting pressures and over time. The challenge is to discriminate between antropogenic-induced changes and the confounding effects of the natural variability of the marine environment.

CONCLUSIONS

HTS methods are commonly used to determine the diversity of complex marine microbial communities and have been proposed as a suitable tool in biodiversity monitoring programs. However, validating their usefulness is crucial for conducting rigorous analyses. Comparison of 454 and Illumina methodologies showed minor differences in the performance of both sequencing methodologies that can in part be attributed to inherent differences in chemistry and sequencing protocols, which may affect the quality of the sequences. Nevertheless, these differences were assigned to very rare OTUs and overall, both platforms provided a comparable view of the marine picoplankton communities. On a taxonomic level, there was very good overlap in the detected phyla between the two methods. The comparative analyses performed suggest that 454 and Illumina data can be combined if the same bioinformatic workflow for describing overall patterns of diversity and taxonomic composition is used. On the other hand, we found that plankton biodiversity surveys have the potential to be used as alternative indicators of environmental status. In particular, using bacterioplankton biodiversity (bacterial richness as well as the ratio between certain bacterial taxa) as an alternative indicator of water quality deserves further investigation. However, these preliminary results have to be further investigated by performing intensive surveys covering wide spatial and temporal scales in order to discriminate between changes resulting from human activities and the natural variability of the marine environment and test whether the identified indices are universally applicable.

AUTHOR CONTRIBUTIONS

Conceived and designed the study: IF, AR, JC, JG, EG; Performed the experiments: IF, CG, AR. Contributed materials: JC, RM, JG, EG; Analyzed the data: IF, CG. Interpreted the data and wrote the paper: IF, CG, AR, JC, RM, JG, EG. All authors reviewed the manuscript.

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Benchmarking DNA Metabarcoding for Biodiversity-Based Monitoring and Assessment

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Characterization of biodiversity has been extensively used to confidently monitor and assess environmental status. Yet, visual morphology, traditionally and widely used for species identification in coastal and marine ecosystem communities, is tedious and entails limitations. Metabarcoding coupled with high-throughput sequencing (HTS) represents an alternative to rapidly, accurately, and cost-effectively analyze thousands of environmental samples simultaneously, and this method is increasingly used to characterize the metazoan taxonomic composition of a wide variety of environments. However, a comprehensive study benchmarking visual and metabarcoding-based taxonomic inferences that validates this technique for environmental monitoring is still lacking. Here, we compare taxonomic inferences of benthic macroinvertebrate samples of known taxonomic composition obtained using alternative metabarcoding protocols based on a combination of different DNA sources, barcodes of the mitochondrial cytochrome oxidase I gene and amplification conditions. Our results highlight the influence of the metabarcoding protocol in the obtained taxonomic composition and suggest the better performance of an alternative 313 bp length barcode to the traditionally 658 bp length one used for metazoan metabarcoding. Additionally, we show that a biotic index inferred from the list of macroinvertebrate taxa obtained using DNA-based taxonomic assignments is comparable to that inferred using morphological identification. Thus, our analyses prove metabarcoding valid for environmental status assessment and will contribute to accelerating the implementation of this technique to regular monitoring programs.

Keywords: Illumina MiSeq, COI barcodes, extracellular DNA, AMBI, biotic indices, macroinvertebrates

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INTRODUCTION

Environmental biomonitoring in coastal and marine ecosystems often relies on comprehensively, accurately, and repeatedly characterizing the benthic macroinvertebrate community (Yu et al., 2012). These organisms are considered a good indicator of ecosystem health and have demonstrated a rapid response to a range of natural and anthropogenic pressures (Johnston and Roberts, 2009). As a result, the macroinvertebrate community has been largely used to develop biotic indices (Diaz et al., 2004; Pinto et al., 2009; Borja et al., 2015), such as the AZTI's Marine Biotic Index (AMBI; Borja et al., 2000), used worldwide to assess the marine benthic status (Borja et al., 2015). Nevertheless, biomonitoring based upon benthic organisms has limitations because species identification requires extensive taxonomic expertise and it is time-consuming, expensive, and laborious (Yu et al., 2012; Wood et al., 2013; Aylagas et al., 2014). The rapid development of high-throughput sequencing (HTS) technologies represents a promising opportunity for easing the implementation of molecular approaches for biomonitoring programs (Bourlat et al., 2013; Dowle et al., 2015). In particular, DNA metabarcoding (Taberlet et al., 2012a) allows the rapid and cost-effective identification of the entire taxonomic composition of thousands of samples simultaneously (Zepeda Mendoza et al., 2015) and the ability to provide a more comprehensive community analysis than traditional assessments (Dafforn et al., 2014), which can enable the calculation of benthic indices in a much faster and accurate way compared to morphological methodologies.

Metabarcoding consists of simultaneously amplifying a standardized DNA fragment specific for a species (*barcode*) from the total DNA extracted from an environmental sample using conserved short DNA sequences flanking the barcode (*primers*; Hajibabaei, 2012; Cristescu, 2014). The obtained barcodes are then high-throughput sequenced and compared to a previously generated DNA sequence reference database from well-characterized species for taxonomic assignment (Taberlet et al., 2012a). In the case of animals, different barcodes such as portions of the small and large subunits of the nuclear ribosomal RNA (18S and 28S rRNA) genes (Machida and Knowlton, 2012) and of the mitochondrial cytochrome oxidase I (COI; Meusnier et al., 2008) and 16S rRNA genes (Sarri et al., 2014) have been proposed for metabarcoding. The COI gene is by far the most commonly used marker for metazoan metabarcoding (Ratnasingham and Hebert, 2013), for which thousands of reference sequences are available in public databases [the Barcode of Life Database (BOLD) contains >1,000,000 COI sequences belonging to animal species] and several amplification primers have been designed [more than 400 COI primers are published in the Consortium for the Barcode of Life (CBOL) primer database].

Several studies have used metabarcoding to characterize the metazoan taxonomic composition of aquatic environments (Porazinska et al., 2009; Chariton et al., 2010; Fonseca et al., 2014; Dell'Anno et al., 2015; Leray and Knowlton, 2015; Chain et al., 2016), and an increasing number of studies have directly applied the approach for environmental biomonitoring purposes (Ji et al., 2013; Dafforn et al., 2014; Pawlowski et al., 2014; Chariton et al., 2015; Gibson et al., 2015; Pochon et al., 2015; Zaiko et al., 2015). Initial studies inferring biotic indices from molecular data show the potential of metabarcoding for evaluating aquatic ecosystem quality (Lejzerowicz et al., 2015; Visco et al., 2015). However, before implementation of metabarcoding in regular biomonitoring programs, this approach needs to be benchmarked against morphological identification so that accurate taxonomic inferences and derived biotic indices can be ensured (Aylagas et al., 2014; Carugati et al., 2015). The accuracy of metabarcoding-based taxonomic inferences relies on the retrieval of a wide range of taxonomic groups from a given environmental sample using the appropriate barcode, primers, and amplification conditions (Deagle et al., 2014; Kress et al., 2015), and on the completeness of the reference database (Zepeda Mendoza et al., 2015). Some attempts have been performed to compare morphological vs. metabarcoding-based taxonomic inferences; yet, results are inconclusive as some studies do not apply both approaches to the same sample and/or have focused on a particular taxonomic group (Hajibabaei et al., 2012; Carew et al., 2013; Zhou et al., 2013; Gibson et al., 2014; Cowart et al., 2015; Zimmermann et al., 2015). A recent study (Gibson et al., 2015) has performed morphological and metabarcoding-based taxonomic identification on the same freshwater aquatic invertebrate samples, but limited their visual identifications to family level. Only two studies (Dowle et al., 2015; Elbrecht and Leese, 2015) have performed a robust benchmarking of metabarcoding using freshwater invertebrates and showed that this technique can be successfully applied to biodiversity assessment. In marine metazoans, all studies have focused only on plankton samples (Brown et al., 2015; Mohrbeck et al., 2015; Albaina et al., 2016). Thus, an exhaustive evaluation of metabarcoding for marine benthic metazoan taxonomic inferences is still lacking.

The use of extracellular DNA (the DNA released from cell lysis; Taberlet et al., 2012b) for biodiversity monitoring is increasingly applied to water (e.g., Ficetola et al., 2008; Foote et al., 2012; Thomsen et al., 2012; Kelly et al., 2014; Davy et al., 2015; Valentini et al., 2016), soil (Taberlet et al., 2012b), and sediment samples (Guardiola et al., 2015; Turner et al., 2015; Pearman et al., 2016). Constituting a significant fraction of the total DNA (Dell'Anno and Danovaro, 2005; Pietramellara et al., 2009; Torti et al., 2015), it is assumed that the taxonomic composition of the free DNA present in the environment reflects the biodiversity of the sample (Ficetola et al., 2008), which would simplify DNA extraction protocols (Pearman et al., 2016) and allow the detection of organisms that are even larger than the sample itself (Foote et al., 2012; Thomsen et al., 2012; Kelly et al., 2014; Davy et al., 2015). Thus, this method appears as a promising cost-effective alternative for macroinvertebrate diversity monitoring, but no robust evidence that the entire macroinvertebrate community can be detected using extracellular DNA exists so far.

The lack of a thorough comparison between morphological and metabarcoding-based taxonomic inferences of marine metazoa and of an evaluation of the use of metabarcoding for marine biotic index estimations prevents the application of metabarcoding in routine biomonitoring programs. Here, we benchmark alternative metabarcoding protocols based on a combination of different DNA sources (extracellular DNA and DNA extracted from previously isolated organisms), barcodes (short and long COI regions), and amplification conditions against benthic macroinvertebrate samples of known taxonomic composition. Additionally, we test the effect of the discrepancies between morphological and DNA-based taxonomic inferences in marine biomonitoring through the evaluation of the molecular based taxonomies performance when incorporated for the calculation of the AMBI and prove the suitability of molecular data based biotic indices to assess marine environmental status.

METHODS

The experimental design followed to compare the performance of molecular and morphological based taxonomic inferences is summarized in **Figure 1**.

Sample Collection and Processing

Benthic samples were collected from 11 littoral stations (sampling depth ranging from 100 to 740 m) along the Basque Coast, Bay of Biscay (Supplementary Figure 1), during March 2013, using a van Veen grab (0.07–0.1 m^2). At each location, after sediment homogenization, one subsample of sediment was taken from the surficial layer of the grab and stored in a sterile 15 ml falcon tube at −80 °C until extracellular DNA extraction (see below). In order to collect the benthic macroinvertebrate community (organism size >1 mm) present in each sample, the remaining sediment was sieved on site through a 1 mm size mesh, and the retained material preserved in 96% ethanol at 4 °C until processing (<6 months). Macroinvertebrate specimens were sorted and identified to the lowest possible taxonomic level based on morphology. Following taxonomic classification, each sample was divided into two identical subsamples by taking equal amount of tissue per taxa for each subsample. Tissues from one subsample were pooled and used for bulk DNA extraction. Each tissue of the second subsample was used for individual DNA extraction (see below).

Extracellular, Individual, and Bulk DNA Extraction

Extracellular DNA was extracted following an optimized protocol (Taberlet et al., 2012b). Briefly, 5 g of each sediment sample were mixed with 7.5 ml of saturated phosphate buffer and an equal volume of chloroform:isoamyl alcohol (IAA). After centrifugation for 5 min at 4,000 g, the aqueous phase was passed through a second round of chloroform:IAA purification and ethanol precipitated before elution of resulting DNA pellet in 100 µl Milli-Q water. For individual and bulk processing, total genomic DNA from each tissue and from the mix of tissues composing each sample, respectively, were extracted using the Wizard® Genomic DNA Purification kit (Promega, WI, USA) in a 125 µl of Milli-Q water final elution. The possible presence of PCR inhibitors in the bulk and extracellular DNA were removed using the Mobio PowerClean® DNA Clean-Up Kit. Genomic DNA integrity was assessed by electrophoresis, migrating about 100 ng of GelRed™-stained DNA on an agarose 1.0% gel, DNA purity was assessed using the Nanodrop® ND-1000 (Thermo Scientific) system and DNA concentration was determined with the Quant-iT dsDNA HS assay kit using a Qubit® 2.0 Fluorometer (Life Technologies). About 20 ng of each individually extracted DNA were used for DNA barcoding of single species (see details below). Subsequently, 5 µl of each individually extracted DNA at original concentration were pooled (hereafter referred as "pooled DNA"). Extracellular, bulk, and pooled DNA were used for PCR amplification and sequencing (see below).

Individual PCR Amplification and Sanger Sequencing

Individual DNA barcoding was performed for the species for which no COI barcode was available in public databases (see **Table 1**, Supplementary Material). The standard 658 bp COI barcode (*folCOI*) was targeted using the dgLCO1490 × dgHCO2198 primer pair (Meyer, 2003). Each individual DNA sample was amplified in a total volume reaction of 20 µl using 10 µl of Phusion® High-Fidelity PCR Master Mix (Thermo Scientific), 0.2 µl of each primer (10 µM), and 20 ng of genomic DNA. The thermocycling profile consisted of an initial 30 s denaturation step at 98 °C, followed by up to 35 cycles of 10 s at 98 °C, 30 s at 48 °C, and 45 s at 72 °C, and a final 5 min extension step at 72 °C. PCR products were considered positive when a clear single band of expected size was visualized on a 1.7% agarose gel. Samples with negative product were further amplified with the mlCOIintF × dgHCO2198 primer pair (Leray et al., 2013) targeting a 313 bp fragment of the COI gene (*mlCOI*). Negative samples were included with each PCR run as external control. PCR products were purified with ExoSAP-IT (Affymetrix) and Sanger sequenced.

PCR Amplification for Library Preparation and Illumina Miseq Sequencing

Indexed paired-end libraries of pooled amplicons were prepared using two nested PCRs from the extracellular, bulk and pooled (mix of 5 µl of individually extracted DNA at original concentration) DNA obtained from each of the 11 collected samples. In parallel, three of the samples were processed per triplicate and considered independently in downstream analysis. For the first PCR, two universal primer pairs with overhang Illumina adapters were used to amplify two different length COI barcodes (the *mlCOI* and the *folCOI*). Three different PCR profiles were used to amplify each COI barcode from the bulk and pooled DNAs (46 and 50 °C annealing temperatures and a touchdown profile), whilst the extracellular DNA COI barcodes were amplified with 46 °C annealing temperature. PCRs were performed in a total volume of 20 µl using 10 µl of Phusion® High-Fidelity PCR Master Mix (Thermo Scientific), 0.5 µl of each primer (10 µM), and 2 µl of genomic DNA (5 ng/µl). The PCR conditions for the two different annealing temperatures consisted on an initial 30 s denaturation step at 98 °C, 27 cycles of 10 s at 98 °C, 30 s at 46 or 50 °C, and 45 s at 72 °C, and a final 5 min extension at 72 °C. For the touchdown profile the PCR conditions consisted on an initial 30 s denaturation step at 98 °C, 16 cycles of 10 s at 98 °C, 30 s at 62 °C (−1 °C per cycle), and 60 s at 72 °C, followed by 17 cycles at 46 °C annealing temperature, and a final 5 min extension at 72 °C (Leray et al., 2013). Negative controls were included with each PCR. Generated amplicons were purified with AMPure XP beads (Beckman Coulter), eluted in 50 µL MilliQ water and used as templates for the generation of the dual-indexed amplicons in the second PCR round following the "16S Metagenomic Sequencing Library Preparation" protocol (Illumina). Purified PCR products were quantified using the Quant-iT dsDNA HS assay kit using a Qubit® 2.0 Fluorometer (Life Technologies) and further normalized for all samples. Pools

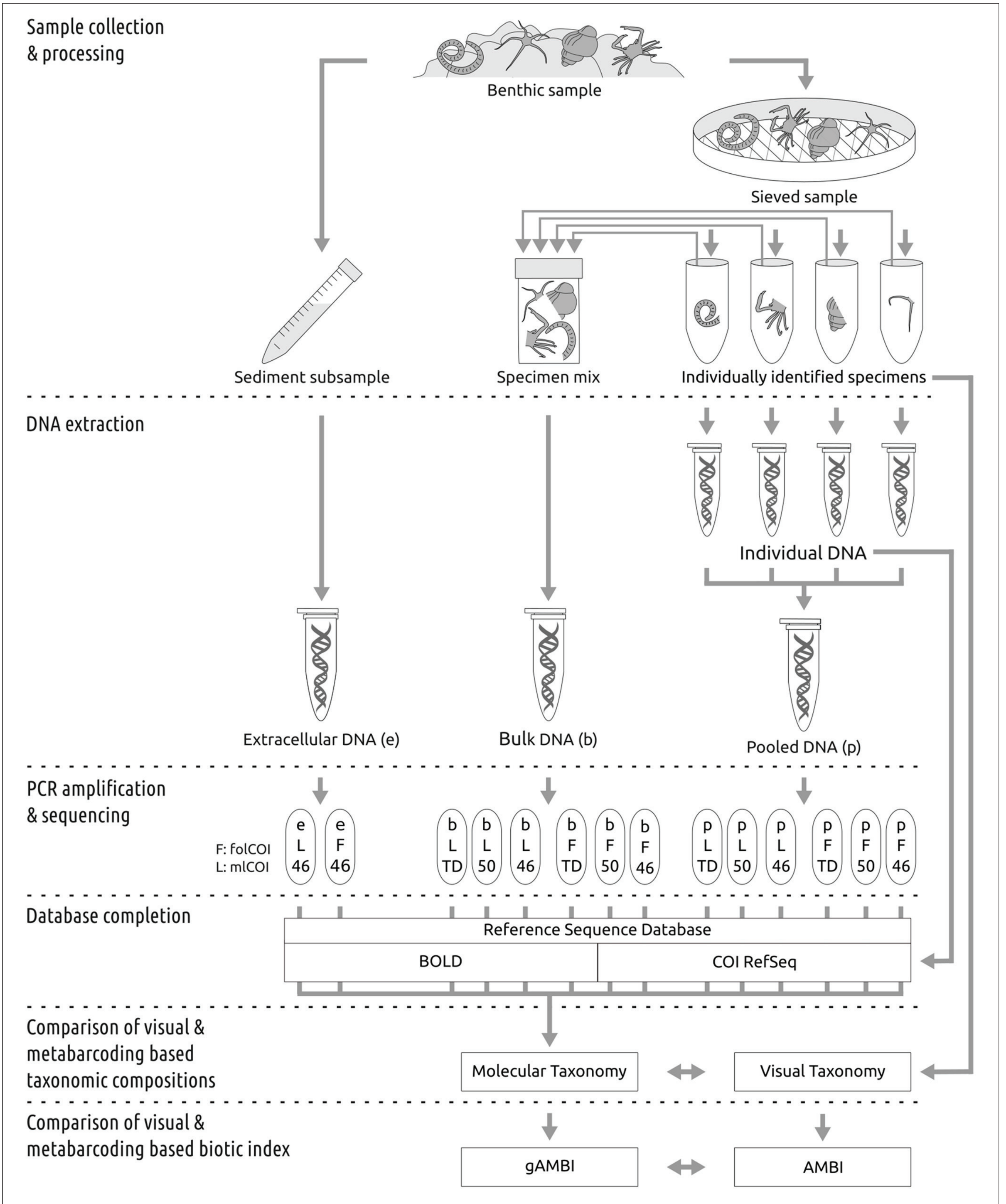

FIGURE 1 | Workflow for sample processing. See Methods Section for detailed explanations.

TABLE 1 | Results from the regression model between traditional and molecularly inferred pa-AMBI values.

Barcode	Condition	R^2	BIAS	RMSE
mlCOI	Bulk DNA 46 °C	0.68*	−0.18	0.28
	Bulk DNA 50 °C	0.49*	−0.21	0.32
	Bulk DNA TD	0.21	−0.22	0.39
	Pooled DNA 46 °C	0.41*	−0.11	0.22
	Pooled DNA 50 °C	0.46*	−0.14	0.23
	Pooled DNA TD	0.03	−0.26	0.40
	Extracellular DNA	0.42*	−0.59	0.83
folCOI	Bulk DNA 46 °C	0.33*	−0.21	0.37
	Bulk DNA 50 °C	0.49*	−0.29	0.43
	Bulk DNA TD	0.07	−0.29	0.49
	Pooled DNA 46 °C	0.02	−0.69	0.83
	Pooled DNA 50 °C	0.01	−0.52	0.59
	Pooled DNA TD	0.01	−0.48	0.57
	Extracellular DNA	0.15	−0.11	0.61

**Significant correlations ($P < 0.05$), TD: touchdown PCR profile.*

of 96 equal concentration amplicons were sequenced using the 2 × 300 paired-end on a MiSeq (Illumina).

DNA Barcode Reference Database

Trace files of Sanger sequences obtained from individual PCR amplifications were edited and trimmed to remove low quality bases ($Q < 30$) using SeqTrace 0.9.0 (Stucky, 2012) and checked for frame shifts using EXPASY (Gasteiger et al., 2003). COI sequences are available in "BCAS project" at BOLD (http://www.boldsystems.org) and in GenBank (accession numbers KT307619–KT307707). To generate our DNA reference database, we retrieved a total of 1,123,601 public COI aligned sequences from 96,641 different taxa from BOLD (October 2014), including the sequences generated in this study (COI RefSeq). After removing duplicates, a total of 505,033 sequences were kept and trimmed to the 658 bp Folmer COI fragment to generate the "BOLD database." A smaller customized DNA reference database was generated using the 4231 sequences corresponding to species included in the AMBI list (see below; available at http://ambi.azti.es) extracted from the "BOLD database" to build the "AMBI database." For the analyses of the *folCOI* reads, the 249 bp not sequenced internal fragment (see below) was removed from these two databases to construct the "BOLD gapped database" and the "AMBI gapped database." The four resulting databases were formatted according to mothur (Schloss, 2009) standards.

Amplicon Sequence Analysis

Demultiplexed reads were quality checked using FastQC (Andrews, 2010) and primer sequences removed using Trimmomatic 0.33 (Bolger et al., 2014). Since the *mlCOI* paired-end reads overlap in 237 bp and the *folCOI* paired-end reads do not overlap, different preprocessing steps are needed for each COI fragment. Forward and reverse *mlCOI* reads were merged using FLASH (Magoč and Salzberg, 2011) with a minimum and maximum overlap of, respectively, 20 bases below and above the expected overlapping region, and the resulting reads were trimmed using Trimmomatic at the first sliding window of 50 bp with an average quality score below 30. The *folCOI* forward and reverse reads were trimmed at 260 and 200 bp, respectively, based on the quality decrease after these positions observed on FastQC plots. Each pair of forward and reverse-complemented reverse read was pasted to create a 409 bp read that corresponds to the *folCOI* barcode without a 249 bp internal fragment. Further details on this new pipeline developed to analyze the universal 658 bp COI barcode which is too long for most HTS applications such as the Illumina MiSeq are detailed elsewhere (Aylagas and Rodríguez-Ezpeleta, 2016). Preprocessed reads from both barcodes were independently analyzed with mothur following the MiSeq standard operating procedure (Kozich et al., 2013). Briefly, sequences with ambiguous bases were discarded and the rest, aligned to the corresponding BOLD and AMBI reference databases. Only those *mlCOI* and *folCOI* reads aligning inside the barcode region and longer than 200 and 300 bp, respectively, were kept. After chimera removal using the *de novo* mode of UCHIME (Edgar et al., 2011), sequences were grouped into phylotypes according to the taxonomic assignments made based on the Wang method (Wang et al., 2007) using a bootstrap value of 90. The sequences that did not return any taxonomic assignment against the BOLD database were blasted against the NCBI non redundant database. Sequences have been deposited in the Dryad Digital Repository (http://dx.doi.org/10.5061/dryad.0sc0s).

Comparison of Morphological and Metabarcoding-Based Taxonomic Compositions

Only taxa representing at least 0.01% of the reads in one station were considered present in the taxonomic composition inferred from molecular data. An in-house script (Supplementary Figure 2) was used to calculate the degree of match between the molecular and morphologically inferred taxonomic compositions of each station. The detection success was normalized for each sample and transformed to percentage of matches (100% of matches means all taxa identified based on morphology have been detected using DNA-based approaches). Differences in mean values of the taxa detection percentages between DNA extraction methods, primers and PCR conditions were examined using a t-test at alpha = 0.05. Patterns of sample dissimilarity were visualized using non-metric multidimensional scaling (nMDS) based on taxa presence/absence and abundance using the Jaccard and Bray-Curtis indices, respectively, obtained using molecular approaches.

Comparison of Morphological and Metabarcoding-Based Biotic Indices

In order to compare morphological and metabarcoding-based biotic indices, we used AMBI, which is a status assessment index based on the pollution tolerances of the taxa present in a sample, with tolerance being expressed categorically into ecological groups (EGI, sensitive to pressure; EGII, indifferent; EGIII, tolerant; EGIV, opportunist of second order; and EGV, opportunist of first order). We calculated the presence/absence morphology-based AMBI (pa-AMBI) and the presence/absence genetics-based AMBI (pa-gAMBI; Aylagas et al., 2014) inferred through DNA metabarcoding of each sample, using the AMBI 5.0 software (http://ambi.azti.es). The relationships among pa-AMBI and pa-gAMBI values were examined using standardized major axis (SMA) estimation (Warton et al., 2006) using the software SMATR (Falster et al., 2003). In order to evaluate the performance of pa-gAMBI for each condition, root-mean-square error (RMSE) and bias were calculated (Walther and Moore, 2005).

RESULTS

Morphological and Molecular Analysis

In total, 138 macroinvertebrate taxa belonging to nine different phyla were morphologically identified in the 11 stations. Representatives of two main phyla, Annelida, and Arthropoda, are present at all stations, with 94 and 21 taxa, respectively, whereas less represented phyla (Mollusca, Chaetognata, Cnidaria, Echinodermata, Nemertea, Nematoda, and Sipuncula) are absent from some stations and include less number of taxa (Supplementary Table 1). Individual DNA barcoding was successful on 61 and 24 of the 106 identified species with no COI barcode in public databases, for which new *folCOI* and *mlCOI* barcodes were generated, respectively, and included in the reference database. Despite this effort to increase the reference database, 21 species remain without barcode because amplification of both barcodes failed.

For each station, two condition combinations were tested for the extracellular DNA (two different barcodes) and six for the bulk and pooled DNAs (two different barcodes and three different PCR profiles). From the 238 samples analyzed, including triplicates performed on three of the stations, 14 had no PCR amplification (see Supplementary Table 2 for clarification on the number of samples produced for molecular analysis). The 224 remaining samples resulted in 16 million reads, from which about 56% passed quality filters and were used for taxonomic analysis (Supplementary Table 2). Of the total reads obtained from extracellular DNA, 71.5 and 73.4% could not be assigned to any metazoan phylum using the customized BOLD database and 24.9 and 25.6% were not assigned to Metazoa for *mlCOI* and *folCOI*, respectively. When blasted against NCBI, the reads obtained using *mlCOI* matched with bacteria (0.6%), non-metazoan eukaryotes (84%), metazoans (12.2%), or did not provide any match (3%), and the reads obtained using *folCOI* matched with bacteria (66.6%), non-metazoan eukaryotes (6%), metazoans (4.2%), archaea (0.05%), or did not provide any match (23.2%). The percentages of non-metazoan reads are much lower for bulk (0.03 and 0.04%) and pooled DNA (0.1 and 0.3%), and the proportion of Metazoa reads with no phylum assigned are lower for *mlCOI* (23.2 and 10.6% for bulk and pooled DNA, respectively) than for *folCOI* (29.94 and 31.6% for bulk and pooled DNA, respectively).

Comparison of Morphological and Molecular-Based Taxonomic Compositions

From the taxonomic inferences obtained using molecular approaches, only macroinvertebrates were considered for sample comparison (e.g., Chordata records were excluded for downstream analysis). The average percentage of recovered taxa (molecular taxonomy matches visual taxonomy) over all stations using different conditions is shown in **Figure 2** (see Supplementary Figure 3 for percentage of recovered taxa considering only species level identification). Matches for taxonomic inferences based on metabarcoding of extracellular DNA are very low (3.4 and 3.1% for *folCOI* and *mlCOI* respectively), with only taxa from three phyla (Mollusca, Annelida, and Nemertea) retrieved (Supplementary Table 3). Results obtained between replicates from the same sample reveal similar taxonomic inferences. No significant differences were observed between the percentage of matches obtained using bulk and pooled DNA ($p > 0.05$). Interestingly, the *mlCOI* barcode outperforms the *folCOI* barcode ($p < 0.05$ for bulk and pooled DNA) and, within the *mlCOI*, the 46 and 50 °C annealing temperatures outperform the touchdown profile both for bulk and pooled DNA ($p < 0.05$). Overall, the best performing condition is the *mlCOI* barcode amplified using 46 °C annealing temperature, which results in a percentage of recovered taxa of 62.4% for all matches and of 76.3% for only matches at species level.

Using molecular approaches we were able to retrieve taxa that had not been morphologically identified. Representatives of Annelida (e.g., *Tubificoides amplivasatus*, *Chloeia parva,* and *Mugga wahrbergi*), Arthropoda (e.g., *Scyllarus arctus* and *Limnoria* sp.), Mollusca (e.g., *Nucula nucleus*, *Galeomma turtoni*, *Thyasira ferruginea,* and *Entalina tetragona*), and Echinodermata (e.g., *Ophiura albida* and *Macrophiothrix* sp.) were solely identified using DNA-based approaches. Moreover, we were able to find taxa belonging to two phyla that were not morphologically identified even at phylum level: two families (Triaenophoridae and Echinobothriidae) and one order (Acoeala) of Platyhelminthes and one family (Hemiasterellidae) of Porifera. As illustrated by the nMDS ordination plot of beta diversity (**Figure 3**), the greatest disparity in macroinvertebrate composition inferred using molecular taxonomy of each station was shown by the extracellular DNA approach.

Comparison of Morphological and Metabarcoding-Based Biotic Indices

The correlation between pa-AMBI and pa-gAMBI values obtained from the taxonomic composition inferences using the AMBI database is shown in **Figure 4**. The pa-AMBI values that best correlate with pa-gAMBI values are those obtained using bulk and pooled DNA approaches at 46 or 50 °C annealing temperatures obtained with *mlCOI* (**Table 1**). Generally, pa-gAMBI values tend to score lower than pa-AMBI values (negative

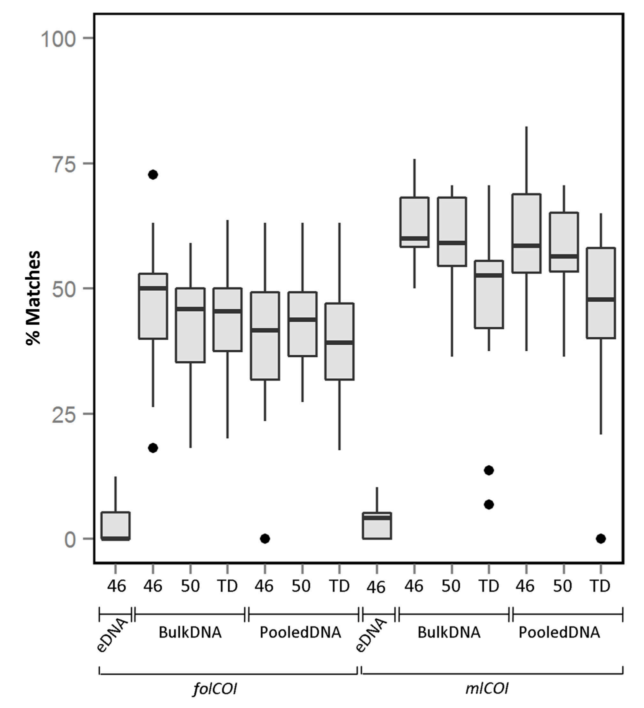

FIGURE 2 | Boxplot showing the percentage of matches obtained between morphological and molecularly inferred taxonomic compositions over all stations. All matches using extracellular DNA (eDNA), bulk and pooled DNA approaches using different PCR conditions (46 or 50 °C annealing temperatures or TD: touchdown profile) for *folCOI* and *mlCOI* barcodes.

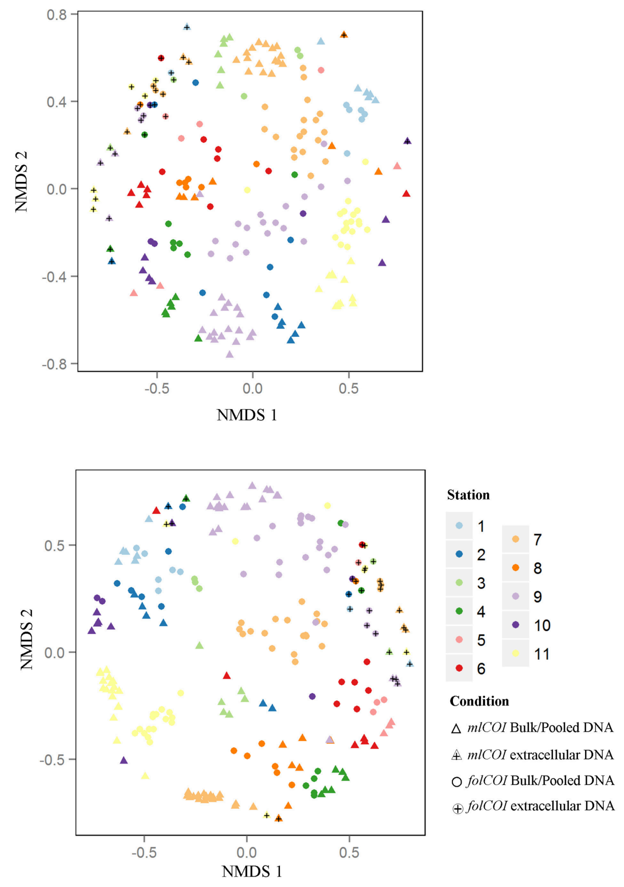

FIGURE 3 | Non-metric multidimensional scaling (nMDS) plots. (Top) Jaccard (presence-absence) and **(Bottom)** Bray-Curtis (abundance) dissimilarities for 32 samples of extracellular DNA and 192 samples of bulk or pooled DNA approaches, from 11 littoral stations for the two barcodes (*mlCOI* and *folCOI*).

bias over all stations). This tendency can be also observed in the variation of the percentage of taxa found belonging to each ecological group obtained using morphological and molecular taxonomic identifications (Supplementary Figure 4). The non-detection of taxa belonging to tolerant and opportunistic ecological groups (III, IV, and V) when using *folCOI*, especially for pooled DNA method, leads to poor correlations between pa-AMBI and pa-gAMBI values.

DISCUSSION

Effect of PCR-Based Analysis Biases on Taxonomic Inferences

Finding the primer pair and PCR conditions that most accurately recover the organisms present in an environmental sample is crucial for a successful application of metabarcoding to biomonitoring. Several studies analyzing the same samples with morphological and molecular taxonomy have been performed so far to benchmark COI based metabarcoding in animals, all focusing exclusively on freshwater or terrestrial macroinvertebrates (Hajibabaei et al., 2012; Carew et al., 2013; Gibson et al., 2014; Dowle et al., 2015; Elbrecht and Leese, 2015) or carried out under morphological identifications limited to high taxonomic levels (Gibson et al., 2015). Thus, studies on marine benthic communities that prove the suitability of DNA-based approaches for environmental biomonitoring are lacking. Using samples of known taxonomic composition, we show that an alternative barcode that targets a shorter region of the COI gene outperforms the 658 bp region that is commonly used for metabarcoding metazoans (Carew et al., 2013; Ji et al., 2013; Dowle et al., 2015; Elbrecht and Leese, 2015; Zaiko et al., 2015). Our data corroborate previous studies unveiling the lack of universality in the COI primers, which is translated to biases during PCR step (Pochon et al., 2013; Deagle et al., 2014). However, the increased performance of the short region, previously demonstrated for individual barcoding on marine metazoans (Leray et al., 2013) and metabarcoding in insects (Brandon-Mong et al., 2015) proves that the *mlCOI* barcode retrieves a high proportion of the morphologically identified taxa. This fact also corroborates the preferred use of small barcodes for metabarcoding, which provide pair-end overlaps on Illumina sequencing and good taxonomic resolution for species identification (Meusnier et al., 2008). Additionally, the *folCOI* barcode returns more reads with no match and metazoan reads not assigned to any specific phylum, which could be attributed to the fact that longer barcodes can accumulate more errors

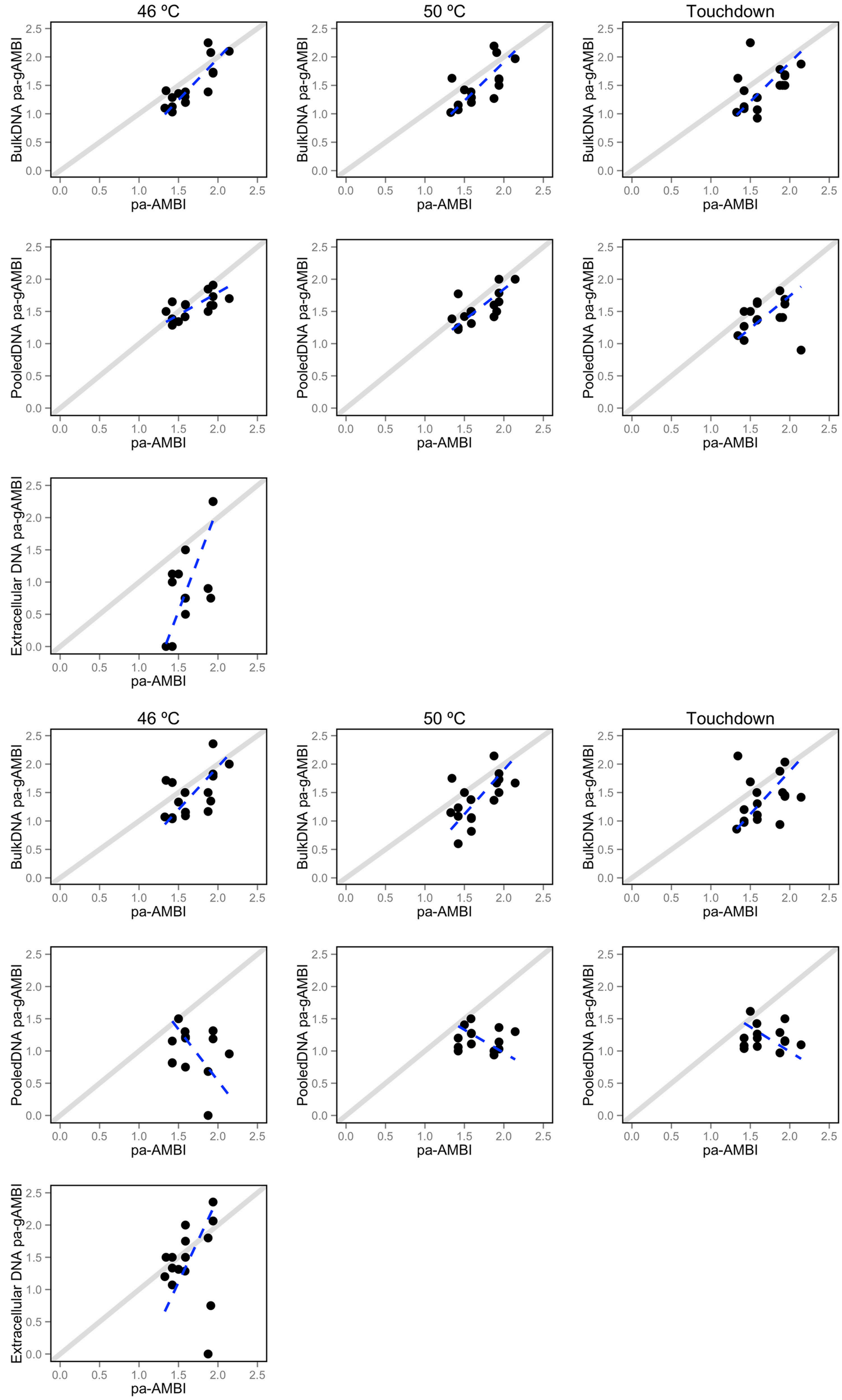

FIGURE 4 | Relationship between pa-AMBI and pa-gAMBI values. For each DNA-based approach (extracellular, bulk and pooled DNA) and PCR condition (46 or 50 °C annealing temperatures or Touchdown profile) displayed separately for each barcode—*mlCOI* (top 3 rows) and *folCOI* (bottom 3 rows). Each dot shows the relationship between the pa-AMBI (x-axis) and pa-gAMBI value (y-axis) for each station. The dotted lines represent the results of model II regression and the diagonal showing perfect correlation between the two observations is depicted.

during the PCR and sequencing processes (Schirmer et al., 2015).

The effect of the PCR annealing temperature has been shown to affect retrieved taxonomic composition in bacterial and archaeal metabarcoding using the 16S rRNA gene (Sipos et al., 2007; Lee et al., 2012; Pinto and Raskin, 2012). Here, we show that the use of inappropriate PCR conditions can also affect the final taxonomic assignment in metazoan metabarcoding analyses. Our results show that a constant low annealing temperature (46 or 50 °C) provides more accurate taxonomic inferences compared to the touchdown profile, which contrasts with previous studies (Hansen et al., 1998; Simpson et al., 2000; Leray et al., 2013). Moreover, it is well-established that the more PCR cycles, the more spurious sequences and chimera are formed during PCR (Haas et al., 2011), which could explain the lower taxa detection rate when using the touchdown profile (which includes five more cycles). Further, the nature of the organisms and their size may bias DNA extraction (i.e., hard shells or chitin exoskeleton can prevent cell lysis and DNA from small organisms can be less effectively extracted). Here, we have ensured that DNA from all organisms is present in the pooled sample by pooling individually extracted DNAs, and show that the results of the pooled DNA and bulk extracted DNA are comparable.

The Use of Extracellular DNA for Biodiversity Estimations

The extracellular DNA-based metabarcoding for biodiversity assessments has the potential of detecting big-size organisms in small samples, which facilitates sampling strategies and could resulting in a more cost-effective approach for environmental biomonitoring (Taberlet et al., 2012b; Thomsen et al., 2012; Thomsen and Willerslev, 2015). Several studies have used extracellular DNA from the water column to detect vertebrates (Ficetola et al., 2008; Thomsen et al., 2012; Valentini et al., 2016) freshwater macroinvertebrates (Goldberg et al., 2013; Mächler et al., 2014) and benthic eukaryotes (Guardiola et al., 2015; Pearman et al., 2016). Yet, so far, this approach has not been proved valid for biodiversity assessment as no comparison with samples of known taxonomic composition has been performed. To our knowledge, only one attempt exists to detect the whole freshwater benthic macroinvertebrate community from extracellular DNA extracted from samples of known composition (Hajibabaei et al., 2012), but the authors used the preservative ethanol as controlled environment containing the free DNA rather than natural scenarios. In our analyses, only a small proportion of the taxa identified using morphological methods are retrieved using extracellular DNA present in the sediment. Indeed, even considering the taxa not identified through morphological taxonomy, the extracellular DNA-based analyses only identify 30 macroinvertebrate taxa over all stations, which is much lower than the total diversity inferred from morphology and from DNA extracted from the isolated organisms. Therefore, the striking differences obtained between morphological and extracellular DNA metabarcoding based taxonomic inferences suggest that further studies are needed before using sediment extracellular DNA as a suitable source for macroinvertebrate biodiversity assessment; yet, more experiments testing the effect of sediment sample size, DNA degradation scenarios, or DNA extraction protocols are required, as it is possible that sampling more deeply in the sediment, or using the water column provides better results, and/or that the optimal DNA extraction procedure has not been employed (Corinaldesi et al., 2005).

Effect Misinterpreting Community Composition in Environmental Biomonitoring

Environmental biomonitoring programs rely on the detection of a wide range of taxonomic groups, which are usually amplified using universal primers (Leray et al., 2013). The abovementioned biases inherent to PCR-based analyses can lead to greater recovery of sequences of some species and the exclusion of others (Elbrecht and Leese, 2015; Piñol et al., 2015). Thus, it is important to see whether in samples containing species from numerous phyla, metabarcoding is also able to retrieve a high proportion of taxa that suffices for environmental monitoring. In general, we show a high percentage of recovery using bulk DNA among the nine different phyla identified using morphological approach. However, in our metabarcoding analyses, some taxa identified using morphological methodologies remain undetected using both short and long COI barcodes, whereas others appear only using metabarcoding. The species exclusively detected using metabarcoding represent potential cryptic species (e.g., *Tyasira flexuosa*/*Thyasira ferruginea* and *Ophiura texturata*/*Ophiura albida*) or unable to be classified based on morphological characters. Further, some additional identified taxa [i.e., two phyla detected from extracellular DNA (Platyhelminthes and Porifera)] may either represent organisms which had been missed by taxonomy based on morphology and metabarcoding from previously isolated organisms due to their small size (<1 mm) or detected due to the fact that the free DNA has been transported from other localities (Roussel et al., 2015).

Consequences of the misinterpretation of the taxonomic composition could result in erroneous biodiversity assessment, which may impede the implementation of DNA metabarcoding in regular biomonitoring programs (Chariton et al., 2015; Cowart et al., 2015; Lejzerowicz et al., 2015; Zaiko et al., 2015). In particular, calculation of biotic indices based on pollution tolerances assigned to the taxa retrieved from the sample (Maurer et al., 1999; Borja et al., 2000) may be affected by the approach used for taxonomic assignment. We show that, despite using the metabarcoding conditions that most accurately detect the morphologically identified taxa, some differences between both approaches are observed. Yet, in general, pa-gAMBI values obtained from metabarcoding analyses provide significant presence-absence community estimations and can be used for calculating biotic indices.

CONCLUSIONS

Representing a promising opportunity to overcome the time-consuming and high cost of traditional methodologies for species identification, it is anticipated that DNA metabarcoding will be routinely used in biomonitoring programs in the near future. Yet, the application of this technique to regular biomonitoring programs requires benchmarking and standardization. Here, we demonstrate through an exhaustive study design that, using the appropriate conditions, metabarcoding presents a great potential to characterize biodiversity and to provide accurate biotic indices. Thus, our findings will contribute to accelerating the implementation of metabarcoding for environmental status assessment.

ADDITIONAL INFORMATION

Accession codes: All Sanger and Illumina generated sequences have been deposited in GenBank (accession numbers KT307619–KT307707) and DRYAD (http://dx.doi.org/10.5061/dryad.0sc0s).

AUTHOR CONTRIBUTIONS

Conceived and designed the study: EA, AB, and NRE. Performed the experiments: EA. Contributed reagents/materials: XI. Analyzed the data: EA and NRE. Interpreted the data and wrote the paper: EA, AB, and NRE. All authors reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmars.2016.00096

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frontiers in Marine Science

ORIGINAL RESEARCH
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Historical Data Reveal 30-Year Persistence of Benthic Fauna Associations in Heavily Modified Waterbody

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Baseline surveys form the cornerstone of coastal impact studies where altered conditions, for example through new infrastructure development, are assessed against a temporal reference state. They are snapshots taken before construction. Due to scarcity of relevant data prior to baseline surveys long-term trends can often not be taken into account. Particularly in heavily modified waterbodies this would however be desirable to control for changes in anthropogenic use over time as well as natural ecological variation. Here, the benthic environment of an industrialized embayment was investigated (Swansea Bay, Wales, UK) where it is proposed to build a tidal lagoon that would generate marine renewable energy from the tidal range. Since robust long-term baseline data was not available, the value of unpublished historical benthos information from 1984 by a regional water company was assessed with the aim to improve certainty about the persistence of current benthic community patterns. A survey of 101 positions in 2014 identified spatially discrete benthic communities with areas of high and low diversity. Habitat characteristics including sediment properties and the proximity to a sewage outfall explained 17–35% of the variation in the community structure. Comparing the historical information from 1984 with 2014 revealed striking similarity in the benthic communities between those years, not just in their spatial distribution but also to a large extent in the species composition. The 30-year-old information confirmed spatial boundaries of discrete species associations and pinpointed a similar diversity hotspot. A group of five common species was found to be particularly persistent over time (*Nucula nitidosa*, *Spisula elliptica*, *Spiophanes bombyx*, *Nephtys hombergii*, *Diastylis rathkei*). According to the Infauna Quality Index (IQI) linked to the EU Water Framework Directive (WFD) the average ecological status for 2014 was "moderate," but 11 samples showing "poor" and "bad" status indicated possible negative impacts of dredge spoil disposal. Generally the study demonstrated the value of historical information for assessing the persistence of benthic community characteristics, while also highlighting shortcomings if raw data is lost and if the historical baseline does not reflect pristine ecological conditions.

Keywords: Swansea Bay, tidal lagoon, baseline, reference conditions, WFD

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INTRODUCTION

Coastal infrastructure such as seawalls, breakwaters, or jetties impact marine ecosystems (Bulleri and Chapman, 2010; Firth et al., 2013). In recent years there has been growing demand to build marine renewable energy devices, contributing to even more infrastructure (Wilson et al., 2010; Binnie, 2016). In order to assess its environmental impacts developers have to carry out baseline surveys of the diversity and composition of benthic communities in the affected area (Franco et al., 2015). However, these are just snapshots of the situation immediately before construction and questionable long-term reference states. Baseline surveys may be affected by short-term natural impacts such as severe storms, extreme temperatures or unusual riverine freshwater input due to heavy rainfall, or anthropogenic impacts such as maintenance dredging (Kröncke et al., 1998; Bolam et al., 2010; Rangel-Buitrago et al., 2016; Robins et al., 2016). For the baseline to be a critical reference point it ought to establish the long-term condition and patterns of the benthic communities. Ideally, environmental monitoring data from statutory bodies or scientific research can be consulted, but long-term data is often scarce or non-existent or the spatial resolution is insufficient to serve as a suitable baseline. In those cases, where present, historical information may provide a valuable source of information. Marine historical ecology contributes profoundly to our understanding of the coastal environment and is increasingly applied in long-term management and policy (Robinson and Frid, 2008; Engelhard et al., 2015). Generally, historic data provides information on past baselines of biological and environmental parameters and enhances our understanding of the effects of anthropogenic disturbances on marine ecosystems and the role played by humans in shaping our coastal habitats (Lotze et al., 2006).

Currently the construction of a tidal lagoon is proposed for Swansea Bay (Wales, UK), a coastal area considered to be a "heavily modified waterbody" under the EU Water Framework Directive (WFD; 2000/60/EC) as a result of coastal protection measures (**Figure 1**). Under the WFD Swansea Bay's predicted ecological quality is classified as "Bad Potential." The proposed lagoon would exploit the tidal range to generate 320 MW using bulb turbines and power 155,000 homes (Waters and Aggidis, 2016). The wall would be 9.5 km long enclosing 11.5 km^2 of foreshore and seabed. As part of the environmental impact assessment (EIA) to inform the planning consent the developer had to carry out a baseline survey, but long-term information about the benthic communities in the area over recent decades was sparse (Harkantra, 1982; Shackley and Collins, 1984). Suitable historical information for a long-term comparison of benthic communities was located in an unpublished report (Conneely, 1988). In 1984 the regional water authority took 172 benthos samples throughout Swansea Bay to fulfill legislative requirements to protect the natural environment from adverse activities and the need to prepare a discharge policy; the area bordering the bay was, and still is, heavily industrialized and there were a number of domestic sewage discharges (Chubb et al., 1980). The survey was a data treasure chest that could be used to assess long-term changes of benthic community patterns. Since 1984 Swansea Bay experienced changes in its anthropogenic use, for example, a major sewage discharge was closed and relocated in 1999 and some areas were used for mussel cultivation, and these activities had had measurable, localized impacts on benthic communities (Smith and Shackley, 2004, 2006). Over the past decades the shipping channels to Swansea and Port Talbot ports as well as the River Neath were regularly dredged, and the material was discarded at a spoil ground in the outer Swansea Bay area (**Figure 1**).

In 2014 the wider Swansea Bay area was surveyed with a sampling design similar to 1984, and the data was analyzed in the same way as reported by Conneely (1988) for the historical study. The raw data from 1984 had been lost, and hence the comparison between 1984 and 2014 was limited to the figures and tables shown in the historic report. Fortunately the author had applied several techniques which are still valid today, including multivariate community analyses, but for the 2014 data additional analytical tools were applied.

The objectives of this study were to

- Identify current benthic communities in Swansea Bay, characterize their spatial identity and species composition and quantify the extent to which the benthic fauna was driven by habitat characteristics.
- Compare current benthic community patterns with those 30 years ago.
- Assess the current environmental status of the benthic community with indicators accepted by the WFD.

MATERIALS AND METHODS

Study Area and Habitat

Swansea Bay is a shallow embayment on the northern coastline of the Bristol Channel (Wales, UK) with depth generally $<$ -20 m Ordnance Datum (OD; Pye and Blott, 2014). It is exposed to severe hydrodynamic forces due to strong winds and tides generated in the Bristol Channel, as well as North Atlantic swells (Allan et al., 2009). Swansea Bay is characterized by a complex patchwork of bottom substrata (Collins et al., 1980). It consists of depositions of poorly sorted, consolidated glacial boulder clay (glacial till), pebbles and cobbles, sometimes mixed with unconsolidated mud and silt as well as mixed sand, silts and clays with associated peats (Culver and Bull, 1980). Marine sediments in the eastern Swansea Bay area are mixed with re-distributed dredge spoils from the Swansea and Port Talbot docks (Culver and Bull, 1980; Shackley and Collins, 1984). Generally, surface sediments are highly temporarily variable depending on storminess, with an increase in the proportion of sand and the exposure of relic gravel deposits after periods of wave exposure and deposition of mud following calm weather (Shackley and Collins, 1984). Water quality is largely influenced by the hydrology of three river catchments that serve Swansea Bay. It is also influenced by the historical and current industrial activity and associated diffuse and point pollution (surface water run-off) toward the eastern side of the bay. The main sewage outfall for the wider Swansea area is located in the center of the inner bay (**Figure 1**).

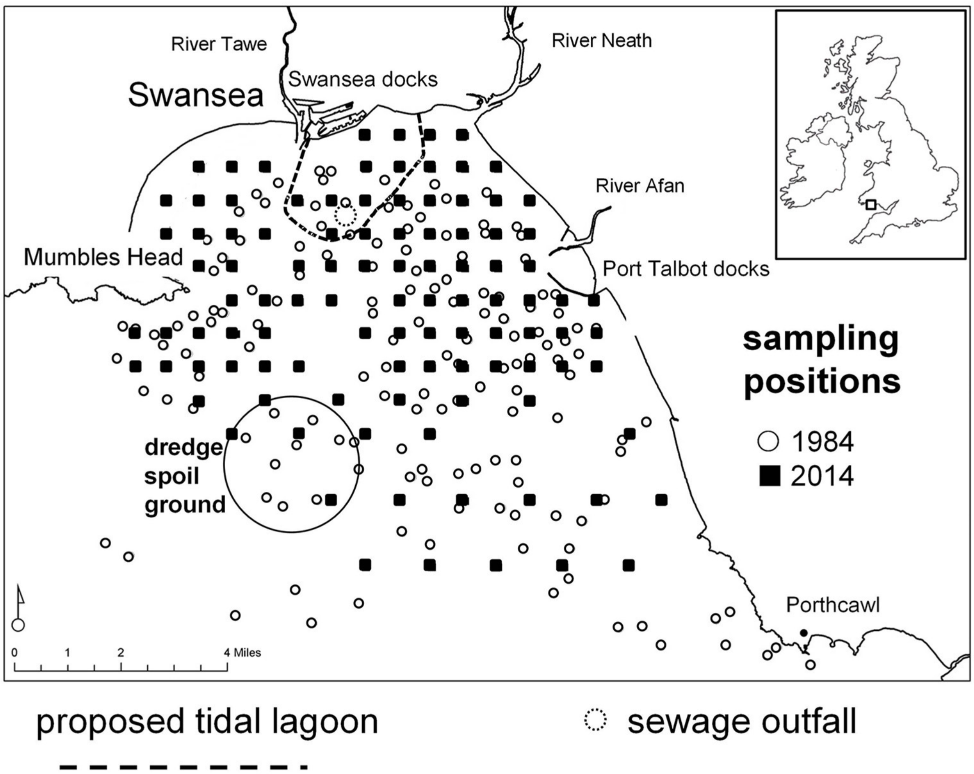

FIGURE 1 | Swansea Bay study area, Wales, UK (51.6197° N, 3.9311° W). Benthos grab-sampling positions in 1984 and 2014 are shown as well as the outline wall of the proposed tidal lagoon, the sewage outfall and the dredge spoil disposal site.

Benthos and Sediments

In 1984 benthos samples had been collected in a 1 km^2 sampling grid in the wider Swansea Bay area, and the same design was adopted in 2014; the reported figure of sampling points in 1984 suggests that the 1 km^2 grid design was only partly realized (**Figure 1**). Altogether 272 sites were visited in 1984, but glacial till limited the number of successful faunal samples to 176. At each position a single sample was taken. In 2014, 129 positions were visited and more samples were taken closer to shore compared with 1984, but fewer samples further offshore due to logistical limitations. Bed rock, boulders, or large shells prohibited the jaws of the benthos grab to close at some positions and here sampling was unsuccessful. Successful benthos samples were retrieved at 101 positions.

Benthos samples were taken with the same 0.1 m^2 Day grab in 1984 and 2014. About 200 g of surface sediment were removed for particle size analysis, and the remaining sediment was washed through a 1 mm sieve. The amount of sediment in each grab sample varied between 3 and 10l. Benthic community parameters were correlated with the amount of sediment per grab sample to test for possible effects of sediment volume on benthic community results, but it had no statistically significant impact (DistLM, $p > 0.05$).

The sieve residue was fixed in 4% formaldehyde and stained with Rose Bengal. All benthic species were sorted from the samples, identified to species level and counted. Sediment samples were air dried and passed through a series of sieves from 2 mm to 63 μm according to the Wentworth–Udden classification scale to determine particle-size distribution. The sediment parameters mean grain size ($\bar{x}$), sorting (σ), skewness (Sk), and kurtosis (K) were calculated with GRADISTAT (Blott and Pye, 2001).

Taking a single sample at each position without replication allowed using available resources to achieve a high resolution in the spatial spread of sampling positions, but it carried the risk that individual sites were not sampled representatively. It was therefore important not to place too much importance on results of individual sampling points, but rather consider groups of sampling points and broader spatial patterns.

Data Analysis

All information of the 1984 study was taken from Conneely (1988). Scanned copies of key figures and tables in the report are provided as Supplementary Material S1.

Cluster Analysis

In 1984 as well as 2014 groups of samples with a similar benthic community were identified by cluster analysis based on Bray–Curtis similarities and group average (Clarke and Warwick, 2001). In order to down-weigh numerically dominant species, the data was $ln(x+1)$ transformed in both studies. For 1984 clusters were identified from the dendrogram shown in Conneely (1988)

(Supplementary Material S1). For 2014 clusters were additionally analyzed by the "similarity profile" (SIMPROF) permutation test in PRIMER (Clarke et al., 2009). This explores the evidence of statistically significant clusters in samples which are *a priori* unstructured.

Indicator Species of Cluster

For the 1984 survey Conneely (1988) determined indicator species of the sample clusters by a set of "pseudo *F*-tests" (Mirza and Gray, 1981), and the method was replicated for the 2014 data. The test is "pseudo F" because it is applied to groups of samples determined by cluster analysis and not to pre-defined, independent sets of samples. Species that are significantly different between clusters in terms of abundance are considered useful discriminators between communities. There are potential pitfalls of such an approach, such as violation of the underlying assumption of normality and multiple comparisons problems, and therefore an increased chance of type I and II errors. The pseudo-*F*-test is rarely used these days to identify species that discriminate groups of samples. Instead, one of the most widely used methods in benthic community studies is SIMPER, which examines the contribution of each species to the average resemblance between sample groups (Clarke and Warwick, 2001). SIMPER additionally determines the contributions of species to the average similarity within a group of samples and hence identifies the species that typify a group; this analysis does, however, not identify discriminator species.

For the 2014 data both methods, SIMPER and pseudo *F*-test, were applied. Identifying indicator species by pseudo-F tests and SIMPER was not directly comparable since SIMPER contrasts pairs of clusters, while pseudo-F compares all clusters simultaneously. It was however possible to broadly assess the resemblance of the species identified by the methods as discriminator species.

Inverse Classification

Associations of species with similar spatial distribution were identified by inverse classification for 1984 and 2014. Two species are thought of as similar if their numbers tend to fluctuate in parallel across sites. Conneely (1988) had performed an inverse cluster analysis based on Bray-Curtis species similarities and reported the species associations for 1984, and the same analysis was carried out for the 2014 data. The Sørenson Index was calculated between species association identified for 1984 and 2014 to assess the similarity between them over time.

Link with Environmental Variables

The extent to which habitat characteristics could explain the multivariate community structure found in 2014 was explored by distance-based linear models (DistLM). The routine allows analyzing and modeling the relationship between a multivariate data cloud, as described by a resemblance matrix, and one or more predictor variables (Anderson et al., 2008; PERMANOVA+ for PRIMER software). DistLM provides quantitative measures and tests of the variation explained by the predictor variables. The sediment properties mean grain size ($\overline{x}$), sorting (σ), skewness (Sk), kurtosis (K), % coarse sand and silt/clay were included as variables as well as depth. Distance of each sampling position to the mouth of the rivers Tawe, Neath, and Afan was entered as a proxy for exposure to freshwater. This was calculated as a cumulative factor weighed according to the size of the catchment: River Tawe 49%, River Neath 32%, River Afan 19%. The distance to the sewage outfall was entered to quantify the impact of nutrient enrichment and point-source pollution. Before DistLM regression was carried out a Draftsman plot was evaluated for multi-collinearity and skewness of data. The Draftsman plot indicated strong correlations between some of the sediment parameters, but r was always below the usual cut-off point of 0.95. Hence, all variables were entered into the model, but it was kept in mind that inter-correlations may render some sediment characteristics redundant as explanatory factors. AIC was used as selection criterion since, unlike R^2, it will not necessarily continue to get better with increasing numbers of predictor variables in the model; a "penalty" term is included in AIC for increases in the number of predictor variables (Anderson et al., 2008). Results of the DistLM were visualized by distance-based redundancy analysis (dbRDA). A vector overlay was added to the ordination diagram of the dbRDA, with one vector for each predictor variable.

Infaunal Quality Index (IQI): Water Framework Directive (WFD) Classification

The IQI was developed to assess the ecological status of the macrobenthic invertebrate infaunal assemblages of sediment habitats in UK coastal and transitional water bodies for the WFD (Phillips et al., 2014). It is a multi-metric index that expresses the ecological health of benthic assemblages as an Ecological Quality Ratio (EQR). It is composed of three individual components: AZTI Marine Biotic Index (AMBI), Simpson's Evenness (1–λ'), and number of taxa (S). To fulfill the requirements of the WFD, the $IQI_{v.IV}$ incorporates each metric as a ratio of the observed value to that expected under reference conditions. For reference conditions sediment properties were entered for each sample. Salinity was standardized to 28 for positions closest to rivers, 31 for other positions in the inner Bay and 32 in the outer bay south of Mumbles Head; salinities were averaged from data provided by Natural Resources Wales.

The IQI was calculated as

$$IQI_{v.IV} = \left(\left(0.38 \times \left(\frac{1 - \left(\frac{AMBI}{7}\right)}{1 - \left(\frac{AMBI_{Ref}}{7}\right)} \right) \right) + \left(0.08 \times \left(\frac{1 - \lambda'}{1 - \lambda'_{Ref}} \right) \right) + \left(0.54 \times \left(\frac{S}{S_{Ref}} \right)^{0.1} \right) - 0.4 \right) / 0.6$$

The resulting EQR ranges from an ecological status "High" (no or very minor disturbance) to "Bad" (severe disturbance; Phillips et al., 2014). It was calculated with the IQI Calculation Workbook

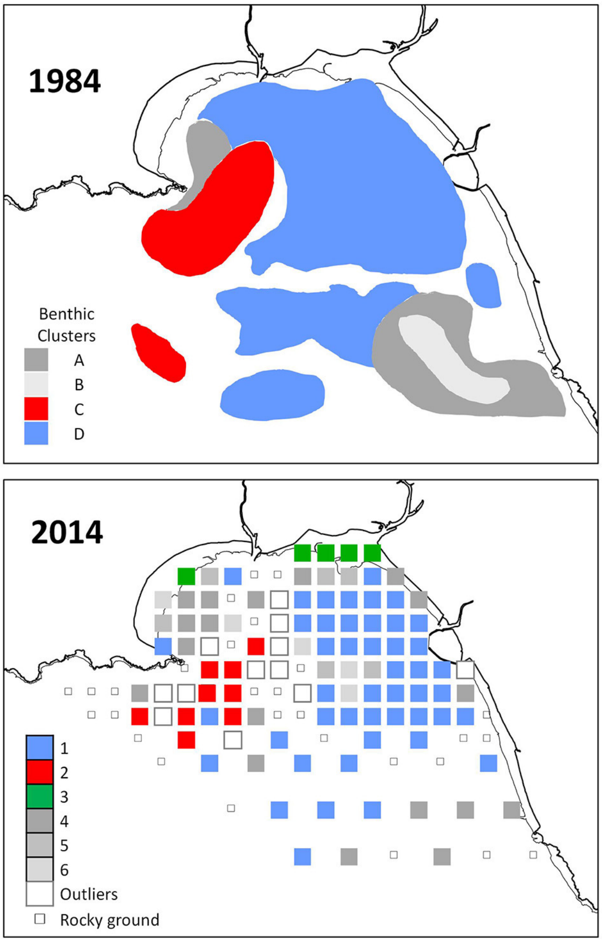

FIGURE 2 | Benthos associations in Swansea Bay delineated by hierarchical cluster analysis based on Bray-Curtis similarities between samples. The 1984 map was adapted from a figure reported by Conneely (1988) (Supplementary Material S1).

UKTAG v.1: update 11/03/2014, which is freely available from the WDF UKTAG webpage.

RESULTS

Benthic Communities

For the 2014 study 188 benthic species were identified from 101 infauna grab samples. The multivariate benthic community analysis comparing all samples classified 21 clusters, and these were grouped into six broader clusters of samples (SIMPROF test based on Bray-Curtis resemblance matrix). The similarity within each of the six clusters was 23–38% (SIMPER).

Cluster 1 covered most of the eastern side of Swansea Bay (**Figure 2**). It was characterized by typical fine-sand species such as the bivalves *N. nitidosa* and *S. elliptica,* the polychaetes *S. bombyx,* and *N. hombergii*, as well as the cumacea *D. rathkei.*

Cluster 2 at the western side of Swansea Bay off Mumbles Head was the most biodiverse cluster with almost four times as many species and five times the number individuals compared

TABLE 1 | Diversity and abundance (0.1 m^{-2}) within clusters delineated by hierarchical cluster analysis for the 2014 benthos survey in Swansea Bay (Figure 2).

	Species richness *S* mean ± SE	Abundance *N* median ± SE	Evenness *J′* mean ± SE
Cluster 1	7.3 ± 0.7	22 ± 12.9	0.8 ± 0.1
Cluster 2	26.2 ± 1.5	115 ± 11.5	0.8 ± 0.1
Cluster 3	3.2 ± 0.6	14 ± 4.3	0.9 ± 0.1
Cluster 4	4.7 ± 0.8	5 ± 3.0	0.9 ± 1.0
Cluster 5	7.2 ± 1.1	10 ± 2.5	0.9 ± 0.9
Cluster 6	4.6 ± 1.2	8 ± 3.4	0.9 ± 0.1

with cluster 1 (**Table 1**). While clusters 1 and 2 had several species in common these were still discriminating the clusters since most species were more abundant in cluster 2, except *N. nitidosa* and *D. rathkei*. Additionally encrusting, sessile, tube-dwelling polychaetes, sipunculids, and phoronids, as well as fully marine species such as the brittle star *Ophiura ophiura* colonized the area grouped as cluster 2 (SIMPER; **Table 2**).

The third cluster was located inshore, characterized by typical lower intertidal to shallow subtidal species such as amphipods of the genus *Bathyporeia* and *Nephtys caeca* (**Table 2**). All other clusters did not have a discrete spatial identity but were interspersed within the other clusters. They were characterized by low numbers of species, which were sub-sets of the three other clusters; cluster 4 additionally contained *Nephtys cirrosa*, cluster 5 the polychaetes *Magelona mirabilis* and *Owenia fusiformis*.

The spatial identity and distribution of the clusters was similar to 1984 (**Figure 2**): cluster 1 in 2014 and cluster D in 1984 both covered the eastern side of Swansea Bay; cluster 2 in 2014 and cluster C in 1984 were located off Mumbles Head; cluster 4 in 2014 was found in similar areas to cluster A in 1984. In 1984 samples had not been taken as far inshore as in 2014 and there were hence no sample positions that could be compared with cluster 3 in 2014. In 2014 too few samples were taken in off-shore areas to make a meaningful comparison with the area of the 1984 cluster B.

There was considerable resemblance in the species composition of individual clusters between 1984 and 2014. Based on the pseudo *F*-test table published in Conneely (1988) the two studies had 26 species in common (**Table 3**); Conneely (1988) reported *F*-tests for 40 species but the full species list was not published for the 1984 study. It was therefore not possible to identify the exact number of common, missing and additional species between studies. Of the 26 species recorded in 1984 as well as 2014, 16 had significant *F*-values in both studies, and 12 species were found in highest numbers in matching clusters.

Similar to 2014, in 1984 the cluster at the eastern side of Swansea Bay was characterized by *N. hombergii*, *N. nitidosa*, *D. rathkei,* and *S. elliptica*. Also, the largest number of species was reported for the cluster C off Mumbles Head; 32 of the 40 indicator species had highest abundances in cluster C (**Table 3**, approx. cluster 2 in 2014). These were mostly polychaetes, tube-dwelling species or sessile species such as *Mytilus edulis*. *Nephtys*

TABLE 2 | Discriminating species between the main groups of samples of the 2014 benthos survey in Swansea Bay (SIMPER).

	$\bar{x}$±SE		Dissimilarity/SD	Contr. to dissimilarity %
	Cluster 1	**Cluster 2**		
Ophiura ophiura	0.2 ± 0.1	9.8 ± 4.0	1.6	4.7
Nephtys hombergii	3.7 ± 0.6	15.8 ± 2.5	1.5	4.6
Nucula nitidosa	16.7 ± 6.4	12.9 ± 4.4	1.4	4.3
Pomatoceros lamarcki	–	6.6 ± 2.9	1.6	4.0
Aphelochaeta marioni	–	9.7 ± 4.7	1.0	3.9
Lumbrineris gracilis	0.1 ± 0.1	2.2 ± 1.4	1.2	3.4
Amphicteis gunneri	–	4.6 ± 1.6	1.3	3.3
Diastylis rathkei	3.0 ± 0.5	0.2 ± 0.2	1.2	2.7
	Cluster 1	**Cluster 3**		
Nephtys caeca	–	5.6 ± 2.4	1.3	12.6
Nucula nitidosa	16.7 ± 6.4	–	1.1	10.9
Diastylis rathkei	3.0 ± 0.5	–	1.0	10.2
Nephtys hombergii	3.7 ± 0.6	–	1.1	8.3
Spiophanes bombyx	11.1 ± 8.5	2.2 ± 1.6	0.9	7.7
Owenia fusiformis	0.2 ±0.1	0.8 ± 2.3	0.8	5.8
Bathyporeia pelagica	–	1.8 ± 1.1	0.7	5.4
Spisula elliptica	3.7 ± 1.3	–	0.8	4.8
Atylus falcatus	1.6 ± 0.5	–	0.6	4.2
Bathyporeia elegans	–	1.8 ± 0.2	0.6	3.0
	Cluster 1	**Cluster 4**		
Nucula nitidosa	16.7 ± 6.4	–	1.1	12.2
Diastylis rathkei	3.0 ± 0.5	0.2 ± 0.2	0.9	11.2
Nephtys hombergii	3.7 ± 0.6	0.2 ± 0.2	1.1	9.2
Nephtys cirrosa	0.4 ± 0.2	1.7 ± 0.3	1.2	8.8
Spiophanes bombyx	11.1 ± 8.5	2.5 ± 2.0	1.0	6.8
Atylus falcatus	1.6 ± 0.5	0.6 ± 0.2	0.8	6.1
Spisula elliptica	3.7 ± 1.3	0.2 ± 0.1	0.8	5.7
Glycera tridactyla	0.7 ± 0.2	0.8 ± 0.2	1.0	5.1

Groups were delineated by hierarchical clustering based on Bray-Curtis sample similarities. Cluster 1 (n = 46) eastern Swansea Bay; Cluster 2(n = 9) western side of Swansea Bay off Mumbles Head; Cluster 3 (n = 6) inshore areas; Cluster 4 (n = 18); ***Figure 2****; mean densities per 0.1 m^2 are shown for clusters.*

cirrosa was the indicator species in the species-poor cluster A in 1984, which matched cluster 4 in 2014.

In comparison with 1984 the mean abundance of *N. hombergii* was lower on the eastern side of Swansea Bay in 2014. Conversely, the average density of *N. nitidosa* was higher in areas off Mumbles Head in 2014 compared with 1984; however, the species' distribution was generally patchy and standard deviations were high (**Table 2**, **Figure 3**). The difference in *F*-values for individual species in 1984 and 2014 supports that mean abundances in clusters differed between the studies. Further, some species were relatively abundant in the 2014 survey but were not reported for 1984, such as the polychaete *Aphelochaeta marioni* or *O. ophiura*.

Species Associations

Inverse cluster analysis identified 5 species associations in 1984 (Conneely, 1988) and 10 associations in 2014. Several associations had common species in 1984 and 2014 (**Table 4**). The greatest similarity (Sørensen Index) was found for the Nucula-association (*N. nitidosa*, *S. elliptica*, *D. rathkei*, *N. hombergii,* and *S. bombyx*). The species were mostly found in the eastern half of Swansea Bay and off Mumbles Head, broadly coinciding with sample clusters 1 and 2 (**Figures 2**, **3**).

Link between Environment and Benthos

Distance-based linear models (DistLM) allowed quantification of the degree to which one or more environmental parameters explained the benthic community structure in 2014; this analysis could not be carried out for the 1984 survey. The overall best model explained 35% of the resemblance in species richness and contained five variables (S: depth, mean grainsize, sorting, % coarse sediment and distance to sewage outfall). Of all entered variables "distance to the sewage outfall" explained most of the variation (6.3%, $n = 101$, $p = 0.0032$) in the data. The best model for the Nucula-association explained 22% of the variation and consisted of six factors: depth, mean grainsize, sorting, % coarse sand, % silt/clay, and distance sewage outfall; individually sediment sorting explained most of the variation (5.5%, $n = 79$, $p = 0.0018$).

For the entire multivariate benthic community matrix containing all species, each of the entered explanatory variables was individually a statistically significant predictor of the multivariate community structure ($n = 101$, $p < 0.005$ for each variables), each explaining 2.6–5.6% of the variation in the benthic community (Supplementary Material S2). The overall best model explained 17% of the variation and contained five factors: mean grainsize, sorting, % coarse sand, distance to sewage outfall, and distance to rivers. Individually sediment sorting explained most of the variation (5.6%, $n = 101$, $p = 0.0001$); distance to the sewage outfall explained 2.5% of the variation ($n = 101$, $p = 0.001$), and distance to rivers 2.4% ($n = 101$, $p = 0.0023$). The model is illustrated in **Figure 4**, where the dbRDA ordination of the benthic community is superimposed by explanatory variables. The dbRDA plot broadly groups the samples similar to the cluster analysis, at least for cluster 1. It ought to be noted that the dbRDA shows just 63% of the fitted variation and therefore captures only part of the model.

Infaunal Quality Index (IQI): Water Framework Directive (WFD) Classification

The majority of samples indicated "moderate" or "good" environmental status according to the WFD classification (**Figure 5**). The IQI in the inner bay was 0.61 ± 0.08 (mean ± sd, $n = 45$) and 0.56 ± 0.15 in the outer bay (mean ± sd, $n = 56$). Both, the outer and inner Swansea Bay fell into the ecological status category "moderate." In the inner bay one sampling location in the vicinity to the sewage outfall was classified as "poor." Eleven samples classified as "poor" or "bad" according to the WFD were in proximity to the spoil disposal site in the outer Swansea Bay area (**Figure 1**).

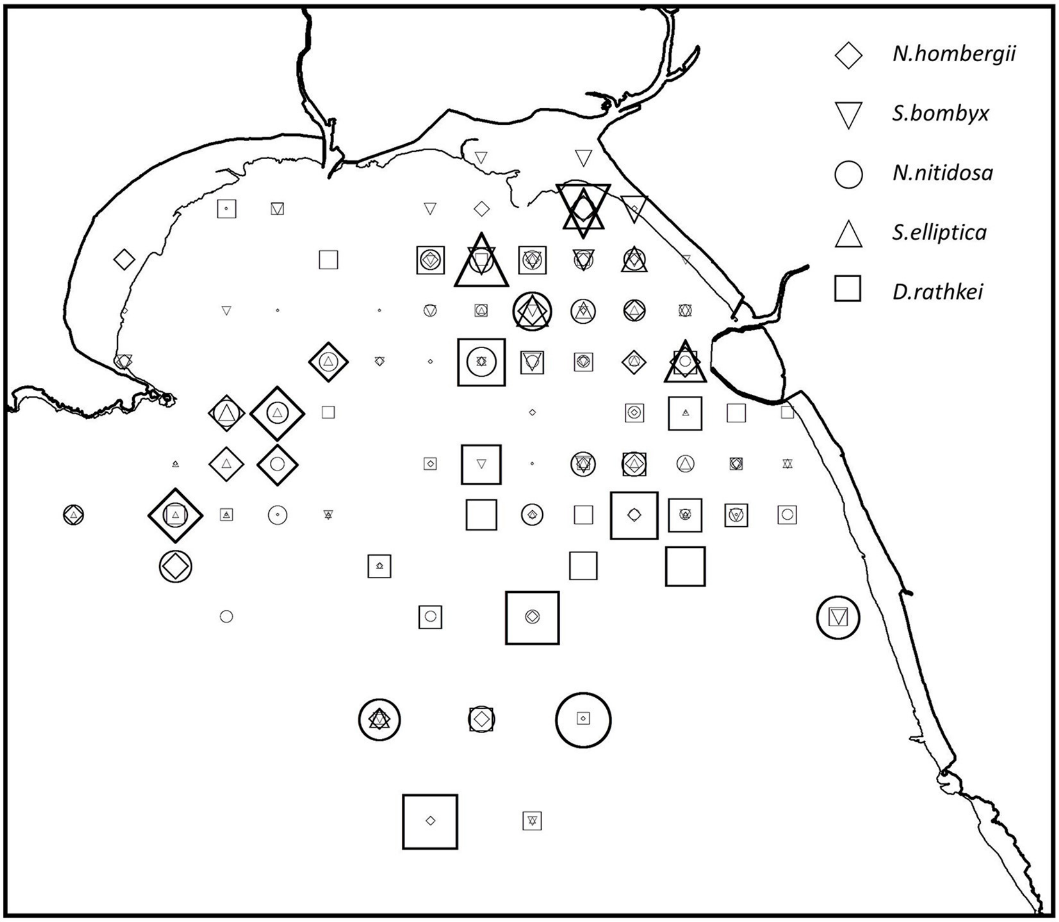

FIGURE 3 | Distribution of species recorded in 1984 and 2014 which were identified by inverse classification as an association with similar spatial trends in both studies. The size of symbols represents the relative abundance of species in the 2014 study.

DISCUSSION

Over the past decades some benthic communities along European coasts changed markedly in response to sea-level rise, invasive species or eutrophication, while others remained relatively unchanged (Hinz et al., 2011; Schumacher et al., 2014; Singer et al., 2016). The benthos of the urbanized Swansea Bay in South Wales (UK) showed strong resemblance in 1984 and 2014, despite changes in anthropogenic use during the past decades. This study provided evidence of striking similarities in the species composition and spatial mosaic of the benthic fauna. Since the two surveys were 30-years apart it is possible that the communities experienced changes during the intervening years. However, published records from before 1984 tie in well with the surveys described here, which suggests that the results may not reflect ephemeral conditions but relatively persistent community patterns (Warwick and Davies, 1977; Harkantra, 1982; Shackley and Collins, 1984). Still, given the uncertainty regarding the nature of the community during the intervening 30 years the term "persistence" is used sensu Grimm and Wissel (1997). According to their definition "persistence" is a stability property that allows for temporal variation in an ecological system which remains essentially the same over time; in contrast, the term "constancy" describes a system that stays unchanged.

In 1984 as well as 2014 a biodiversity hotspot was identified in an area further off-shore in mixed sediment and rocky grounds off Mumbles Head, a carboniferous limestone headland (**Figure 2**, **Table 1**). Reasons for the diversity-promoting conditions in that area were not obvious. Tidal flow velocities are exceptionally high around Mumbles Head where they are enhanced by an anticyclonic gyre, and generally tidal current speed and species richness are negatively correlated in sedimentary habitats (Warwick and Uncles, 1980; Rees et al., 1999; Pye and Blott, 2014). However, the current speed may not be the direct challenge for benthic species but rather the associated sediment movement (Warwick and Uncles, 1980). It is possible that off Mumbles Head high current velocities coincide with relatively stable substratum due to its mixed nature of glacial deposits and marine sediments with low sedimentation rates (Pye and Blott, 2014). This provides hard substratum and environmental conditions suitable for sessile species vulnerable to sedimentation and erosion. Further, tidal currents transport

TABLE 3 | Indicator species of sample clusters in 1984 and 2014 (Figure 2) determined by pseudo *F*-test.

	1984					2014			
	***F*-value**	**A**	**B**	**C**	**D**	***F*-value**	**1**	**2**	**4**
Nephtys hombergii	57.8	0.1		2.3	4.9	13.3	1.1	2.6	
Lumbrineris gracilis	56.2			1.3	0.1	16.5	0.1	1.3	
Nephtys cirrosa	52.7	2.0	0.3	0.1	0.1	9.8	0.2		0.9
Pomatoceros lamarcki	48.0	0.1		1.5		24.5		1.5	
Cirriformia tentaculata	35.9	0.1		2.2	0.1	0.2[(a)]	0.1		
Notomastus latericeus	27.8			1.0	0.1	7.4	0.3	1.2	
Protodorvillea kefersteini	22.4			0.5		5.8	0.1	0.7	
Nucula nitidosa	21.1	0.1		0.1	3.3	9.6	1.5	2.2	
Polycirrus sp.	17.8		0.1	0.3		12.5	0.1	0.9	
Amphicteis gunneri	15.1			0.3		26.0		1.3	
Golfingia elongata	15.1			0.2		1.9[(a)]		0.1	
Phoronis sp.	12.4			0.2	0.1	1.1[(a)]	0.1	0.2	
Glycera tridactyla	11.9	0.1		0.9	0.1	0.9[(a)]	0.3	0.2	
Kefersteinia cirrata	11.9			0.2		1.9[(a)]		0.2	
Lagis koreni	9.3			0.2	0.1	0.3[(a)]	0.1		
Mytilus edulis	9.2			0.9		2.4	0.1	0.2	
Sthenelais boa	9.0			0.3	0.1	10.2		0.6	
Lepidonotus squamatus	8.4			0.2		3.8		0.4	
Eumida sanguinea	7.8			0.2	0.3	1.9[(a)]		0.1	
Sabellaria spinulosa	7.4			0.3	0.3	4.2		0.5	
Diastylis rathkei	7.1	0.1			0.6	9.4	1.1	0.1	
Gattyana cirrosa	5.7			0.1		1.9[(a)]		0.1	
Scoloplos armiger	5.4	0.1		0.4	0.1	0.5[(a)]	0.1	0.1	
Golfingia vulgaris	5.4			0.1		10.6	0.2	1.1	
Urothoe brevicornis	5.2	0.2				1.6[(a)]		0.1	
Spisula elliptica	4.1	0.1	0.1	0.1	0.6	2.8	0.7	0.7	

All tests significant ($p < 0.05$) except those marked with[(a)]. F-values and means of ln(x+1) transformed abundances are shown for species in clusters; only clusters 1,2, and 4 of the 2014 survey had indicator species in common with 1984 and are shown in this table. Clusters with similar spatial identities were A/4, C/2, and D/1.

large quantities of plankton from the inner bay area. This favors suspension and filter feeders and would explain the diverse sessile polychaete fauna, including several tube-dwelling species as well as sipunculids and phoronids, leading to higher diversity and abundance than elsewhere in the bay. While overall community patterns persisted over time, there was evidence of changes in density of individual species. These ought to be interpreted with caution. In Swansea Bay densities of individual species change dramatically not just seasonally, but from month to month and annually, and these are therefore unlikely indicators for long-term changes (Shackley and Collins, 1984; Conneely, 1988; Smith and Shackley, 2004, 2006).

In the 1984 and 2014 surveys a group of five species was prevalent: the bivalves *N. nitidosa* and *S. elliptica*, the polychaetes *N. hombergii* and *S. bombyx,* and the cumacean *D. rathkei* were grouped as species that showed overlap in their distribution (**Figure 3**). The species were found in both main benthic clusters and occurred in large parts of Swansea Bay. They are adapted to living in mobile sediments and coping with erosion and sedimentation, and it seems plausible that this group of species persisted over time because they can tolerate the rigor of the environment (Valentin and Anger, 1976; MarLIN, 2016). Their distribution was significantly linked to sediment properties. Generally, benthic monitoring can be onerous because of the taxonomic expertise necessary to identify large numbers of invertebrate species, and it may be possible to speed up the process by focusing on this group of species. This could provide a rapid indication of spatial change in the benthic community; it would though preclude conclusions about changes in biodiversity.

Distance-based linear models indicated that the composition of the Swansea Bay benthic fauna was significantly linked with sediment properties, the proximity to rivers and the sewage outfall. The close relationship of benthic organisms with sediment characteristics has long been established (Gray, 1974), and in the Bristol Channel and Swansea Bay area faunal associations were shown to be directly related to tidally-averaged bed shear stress, which provided evidence for the physical control of the benthic communities (Warwick and Uncles, 1980). The broad spatial pattern of sediment distribution remained identical over the past 30 years (Harkantra, 1982; Pye and Blott, 2014). However, the 1984 and 2014 studies also highlighted that the traditional method of grab sampling to assess benthic fauna and substratum may not be entirely appropriate for an area

TABLE 4 | Similarity of species associations in 1984 and 2014 (Sørensen Index).

→ 2014 / ↓ 1984		3 *B. pelagica* *N. caeca*	3 *L. koreni*	5 *C. tentaculata* *S. armiger* *M. edulis* *P. muelleri* *Urothoe sp.*	5 *N. nitidosa* *D. rathkei* *N. hombergii* *S. bombyx* *S. elliptica*	5 *G. tridactyla*	11 *G. cirrosa* *L. squamata* *L. gracilis* *P. kefersteini* *S. spinolosa* *K. cirrata*	11 *A. gunneri* *G. vulgaris* *N. latericeus* *P. caliendrum* *P. lamarcki*
7	*Diastylis rathkei* *Nephtys hombergii* *Nucula nitidosa* *Spiophanes bombyx* *Spisula elliptica*				0.83			
28	*Lagis koreni* *Cirriformia tentaculata* *Scoloplos armiger* *Glycera tridactyla* *Gattyana cirrosa* *Lepidonotus squamata* *Lumbrineris gracilis* *Protodorvillea kefersteini* *Sabellaria spinolosa* *Amphicteis gunneri* *Golfingia vulgaris* *Notomastus latericeus* *Polycirrus caliendrum* *Pomatoceros lamarcki*		0.06	0.12		0.06	0.26	0.26
6	*Mytilus edulis* *Phoronis muelleri* *Kerfsteinia cirrata*			0.36			0.12	
3	*Bathyporeia pelagica* *Nephtys caeca* *Urothoe sp.*	0.67		0.25				

Associations were determined for each year by inverse cluster analysis based on Bray-Curtis similarities of ln(x+1) transformed abundances of species. Species jointly found in 1984 and 2014 are listed here; the total number of species in each cluster is shown above and in front of the 2014 and 1984 clusters of each species association. The darker the shading the greater the similarity between associations.

with considerable glacial deposits, because the grab fails in rocky grounds. The coarse glacial material was not sampled representatively and some of the unexplained variation in the data is likely to stem from ignoring the impact of rocky substratum. Our understanding of the benthic ecology in areas with a mosaic of marine sediments and glacial till would improve by applying additional methods such as dredging and underwater video or stills. Further, the benthic models could be improved by more detailed, high resolution information about salinities in Swansea Bay. Distance to three rivers was a significant factor in explaining variation in benthic community characteristics, and it is possible that areas close to the rivers are at least temporarily subjected to full estuarine conditions (Heathershaw and Hammond, 1980).

The sewage outfall was also significantly linked with the benthic community composition, suggesting that this point-source pollution affected the fauna. Invertebrates in heavily modified waterbodies in the vicinity of urban centers can be significantly impacted by an altered food chain, caused by higher nutrient concentrations from domestic and industrial sewage (Puccinelli et al., 2016). This can translate into a compromised ecological status, particularly if it is linked to oxygen depletion (Borja et al., 2009). However, while the distance to the sewage outfall was a statistically significant factor explaining 2–6% of the variation, it was generally part of a group of 5–6 habitat characteristics that best explained larger portions of the overall variation of the benthic community structure.

Infauna Quality Index (IQI) and Ecological Status

The EU WFD water body classification suggests that Swansea Bay has "bad potential," partly because of possible constraints in the distribution of invertebrates due to coastal defense infrastructure and diffuse source pollution. This study showed that in 2014 the average ecological status of Swansea Bay fell into the category "moderate" in terms of its invertebrate fauna. The inner bay in particular was predominantly classified as "moderate" or "good" ecological status (**Figure 5**). A single location in the inner bay was categorized as "poor." The site was in close proximity to the current sewage outfall, which would be a plausible explanation for the poor ecological status (Borja et al., 2006). However, since this was just a single sample the result needs to be viewed with caution, and more replicate samples would be needed to

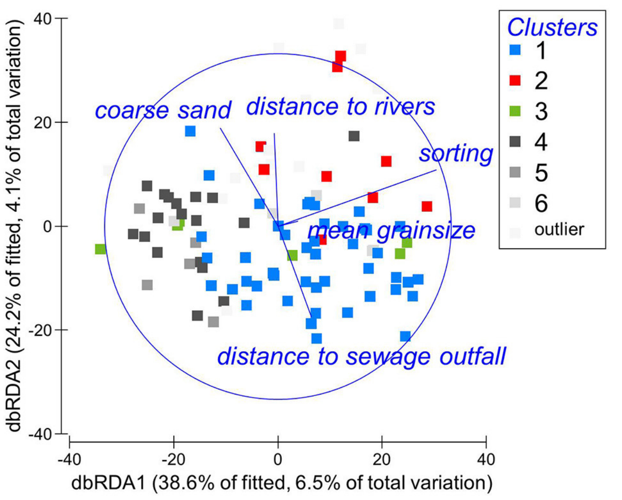

FIGURE 4 | dbRDA ordination of the fitted model for the benthic community of Swansea Bay, based on Bray-Curtis similarities after ln(x+1) transformation of abundances. Habitat characteristics significantly linked to the variation in the data are superimposed.

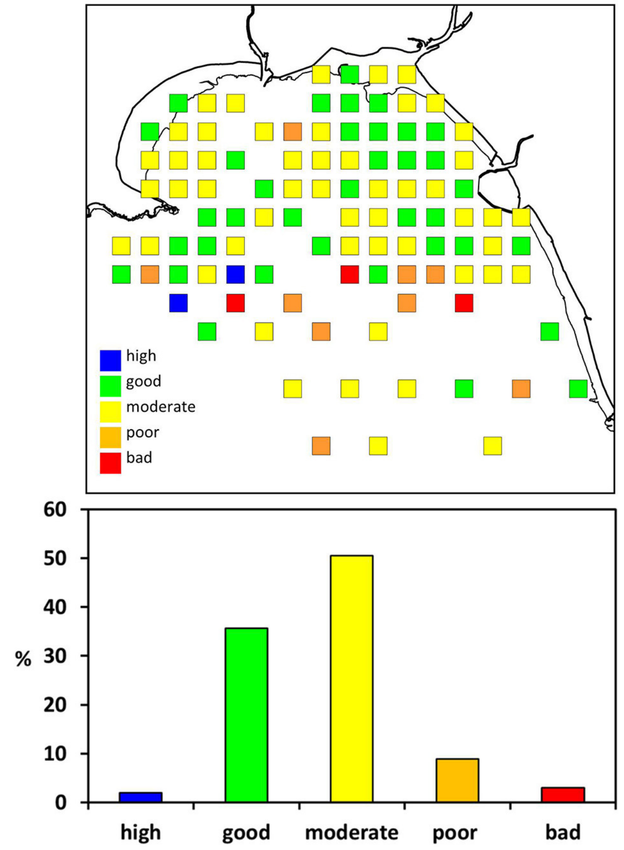

FIGURE 5 | Water Framework Directive (WFD) classification of benthic infauna in Swansea Bay according to the Infauna Quality Index (IQI). It is a combination index based on three individual components: AZTI Marine Biotic Index (AMBI), Simpson's Evenness (1-λ′), and number of taxa (S).

verify the finding. Similar to the benthic community models, the WFD classification for the area would also benefit from more accurate salinity data. The impact of salinity on multimetric parameters is recognized and following from this the importance of geographical separation of areas according to environmental conditions when implementing the WFD (Fleischer and Zettler, 2009). This is however particularly challenging in a relatively small area such as Swansea Bay with spatially and temporarily widely fluctuating salinities.

Eleven samples from the outer bay indicated "poor" or "bad" ecological status. A possible explanation is the vicinity of the dredge spoil ground, used to discard material from maintenance dredging of three shipping channels in Swansea Bay (**Figure 1**). The spoils may either directly impact the benthic community at the disposal site, or sediments may be transported over a wider area, explaining the west-to-east spread of sites with poor ecological status. Dredging and spoil disposal generally increases turbidity, changes sediment composition and mobilizes toxic materials such as heavy metals (Marmin et al., 2014). The nature of the impact of disposing dredge spoils on the benthic fauna varies with site specific environmental factors such as wave exposure and sediment dynamics (Roberts and Forrest, 1999; Bolam et al., 2010). The management of dredging and disposal of spoils would also be of relevance for new infrastructure projects, including the proposed Tidal Lagoon Swansea Bay, since maintenance dredging may be necessary after operation commences. With improving discharge management the risks decrease, but there is considerable uncertainty about the behavior of dredge spoils, and the impact on the ecology of affected areas merits closer investigation.

There was little resemblance between the pattern of the WFD ecological status classifications and the benthic community patterns identified by multivariate community analysis. For example, the biodiversity hotspot off Mumble Head was not generally categorized as having high ecological status. This precluded extrapolation of the 2014 ecological status assessment to 1984: while the broad benthic community patterns may have been similar in 1984 and 2014 it does not necessarily follow that the ecological status was similar too. Long-term studies of the sensitivity and robustness of benthic indicators to natural variability suggested that multimetric parameters such as the IQI will not just respond to anthropogenic impacts which they were designed for, but also to natural variation and disturbance, for example cold winter events and gradual changes in the climate regime (Kröncke and Reiss, 2010). They are however more robust against seasonal and interannual variability than univariate diversity indices. In the 1984 and 2014 comparison of Swansea Bay both natural long-term change as well as changed anthropogenic pressure was intertwined. Had there been significant differences in the benthic community structure, it would have been challenging to disentangle natural and anthropogenic effects. This highlights the importance of not only temporal reference conditions, but also spatial reference states (Borja et al., 2012).

Although this study precluded analyzing temporal differences in ecological conditions, it seems plausible that the ecological status may have changed over time in Swansea Bay. For example, Conneely (1988) suggested that the 1984 benthic fauna was affected by high concentrations of heavy metals in sediments from industrial and sewage discharge (Chubb et al., 1980). In 1999 the sewage treatment and discharge location was moved from the western bay to its current central position (**Figure 1**), triggering a shift in benthic diversity from filter-feeders to deposit feeders in the immediate vicinity of the old sewage pipe (Smith and Shackley, 2006). Further, the start of a commercial mussel lay in the western bay led to a localized increase in carnivores and deposit feeders, but also to an overall decrease in species richness within the mussel bank (Smith and Shackley, 2004). While these changes in anthropogenic use seem not to have altered the broad community patterns over the past 30 years, they are likely to have changed the ecological status in pockets of Swansea Bay.

The historical use of Swansea Bay highlights that the 1984 baseline did not represent a pristine state at which anthropogenic effects could be considered to be negligible (Collins et al., 1980). We know, for example, that about a century ago the area had thriving oyster beds (Shackley et al., 1980). Similar to other European stocks populations declined through overfishing, untreated sewage discharge, heavy metal contamination and shellfish disease (Laing et al., 2006). Despite improvements in water quality and the absence of commercial oyster dredging for decades, the stocks have not recovered, and hence, the anthropogenic activities a century ago may have changed the system beyond natural recovery. Further, coastal defense and infrastructure development in the bay severely modified the bay for over a century. Port Talbot Harbor or the Swansea Dockland/Tawe dredge channel create surrogate headlands which affect localized sediment movement (Thomas et al., 2015). This needs to be taken into account for environmental management, particularly when determining targets and reference conditions.

CONCLUSIONS

This study provides further evidence of the value historical data can add to marine and coastal management, particularly if the repeat surveys are standardized to the historic methodology and complemented with contemporary techniques. On balance this approach maximizes the power of the comparison, although it may not capitalize on all currently available survey techniques. While this study emphasized the opportunities of historical data, it also grappled with limitations of using sub-standard information. The aspiration remains to determine meaningful reference conditions or baselines that can be repeated to track change (Borja et al., 2012). Generally this study highlights the importance to store raw survey data and make them available for future research in public archives. For the development of an infrastructure project such as the tidal lagoon in Swansea Bay this study offers a baseline of spatial benthic diversity patterns and provides information about key species and their relationship with the habitat. While significant environmental variables affecting the benthic community composition were identified, much of the spatial variation in the fauna remained unexplained. In order to improve models, more accurate and detailed information about freshwater input and salinities needs to be generated. The impact of glacial till on the benthic community needs attention, and this study suggested that for areas with a mosaic of marine sediments and glacial deposits traditional sediment property measures may be poorer indicators of the hydrodynamic regime than elsewhere. Direct values of current speed as well as wave exposure and sediment transport could improve the benthic models. Since the proximity of the sewage outfall was a significant contributing factor in explaining benthic characteristics, it would be advisable to measure oxygen concentration in sediments more accurately as a possible explanatory factor linked with nutrient enrichment. Importantly, this study suggests that dredge spoil disposal may affect the current ecological status of the benthic community, and future studies ought to focus on the behavior of dredge spoil disposals in the outer Swansea Bay in order to understand processes that may affect the benthic fauna.

The comparison with a 30 year old baseline removed some uncertainty about the temporal variability of the benthic communities and confirmed that current associations are unlikely to be ephemeral but instead reflect persistent patterns. The severe natural environmental conditions in this heavily modified waterbody appear to have overshadowed localized changes linked with anthropogenic use over the past decades when assessing the area on a larger spatial scale. However, the 1984 baseline portrayed an already highly anthropogenically impacted situation as a result of industrial activities for over a century and did not represent a pristine ecological state. Historical data are therefore not necessarily suitable for setting future targets regarding the environmental status and for assessing if an area is as expected under prevailing conditions, as required, for example, by the Marine Strategy Framework Directive (MSFD, 2008/56/EC; European Commission, 2008). Such a task may be particularly challenging an areas such as Swansea Bay, which have been subjected to century-long anthropogenic impact.

DATA ACCESSIBILITY

This paper highlights the importance of data accessibility and the author strongly supports public availability of raw data. The data of this study will be made publically available through an appropriate public archive once the paper is accepted for publication.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmars.2016.00141

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Need for monitoring and maintaining sustainable marine ecosystem services

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Increases in human population and their resource use have drastically intensified pressures on marine ecosystem services. The oceans have partly managed to buffer these multiple pressures, but every single area of the oceans is now affected to some degree by human activities. Chemical properties, biogeochemical cycles and food-webs have been altered with consequences for all marine living organisms. Knowledge on these pressures and associated responses mainly originate from analyses of a few long-term monitoring time series as well as spatially scattered data from various sources. Although the interpretation of these data can be improved by models, there is still a fundamental lack of information and knowledge if scientists are to predict more accurately the effects of human activities. Scientists provide expert advices to society about marine system governance, but such advices should rest on a solid base of observations. Nevertheless, many monitoring programs around the world are currently facing financial reduction. Marine ecosystem services are already overexploited in some areas and sustainable use of these services can only be devised on a solid scientific basis, which requires more observations than presently available.

Keywords: biodiversity, ecosystem trends, eutrophication, food-webs, global change, ocean acidification, ocean governance, overfishing

INTRODUCTION

The last 10,000 years, known as the Holocene, have been a relatively stable period in earth's climate history (Petit et al., 1999), but recently human activities have become the main driver of environmental change at the local as well as global scale (Rockström et al., 2009). Humans have significantly altered the biogeochemical cycles on earth (Vitousek et al., 1997); something thought impossible just a few decades ago. Burning of fossil fuels, deforestation, mining, and other activities have increased the concentration of CO_2 in the atmosphere and ocean, elevating the greenhouse effect with rising temperatures as consequence. So far, the oceans have managed to store three times as much heat as the atmosphere (Levitus et al., 2001) and absorb about one third of the human-induced CO_2 emitted into the atmosphere (Steffen et al., 2007). However, recent studies suggest that the ocean's buffer capacity might decrease with further warming (Gruber et al., 2004).

Industrial nitrogen fixation and phosphate mining as well as fossil fuel burning have mobilized nitrogen and phosphorus (Vitousek et al., 1997). Humans have almost doubled the supply of nitrogen from the atmosphere to land, leading to an increased release of the greenhouse gas N_2O (Gruber and Galloway, 2008). Phosphate demands for agriculture have increased phosphorus inputs to the biosphere by factor of almost four (Falkowski et al., 2000). Nutrients applied to land as fertilizers are partly lost to the aquatic environment, eventually the ocean, where they stimulate production of organic matter, a process known as eutrophication (Nixon, 1995). One of the most deleterious effects of eutrophication is the development of hypoxia (Carstensen et al., 2014), having strong ramifications on nutrient biogeochemical processes (Diaz and Rosenberg, 2008; Conley et al., 2009).

Human demand on fish has significantly reduced populations of marine top predators (Pauly et al., 1998), altering the flow of energy through food-webs and eventually leading to ecosystem collapses (Jackson et al., 2001). Fisheries landings have increased by more than 50% from 1970 to 2005 (Duarte et al., 2009) and the number of unsustainable fisheries is growing (Vitousek et al., 1997). In addition to reducing the overall population of marine top predators, overfishing has also selected toward smaller populations by removing the largest individuals (Jackson et al., 2001). It is possible that overfishing may exacerbate effects of eutrophication through trophic cascades, disrupting the normal flow of energy through marine food-webs (Scheffer et al., 2005). Another facet of altered energy flows is the global loss of biodiversity caused by overfishing, pollution, and habitat destruction reducing ocean ecosystem services (Worm et al., 2006).

Human pressures on marine ecosystems have increased recently to an extent where every area of the oceans is affected to some degree, although the human footprint is largest in the coastal zones with a high population density (Halpern et al., 2008). The multiple pressures of human activities have eroded the capacity of marine ecosystems to provide services benefitting humans. The oceans no longer constitute an infinite reservoir of natural resources that humans can exploit unconcerned. Therefore, science has an important role in identifying problems as well as their solutions, and conveying this knowledge broadly to the public and particularly, decision makers (Levin et al., 2009).

ASSESSING HUMAN IMPACTS ON MARINE ECOSYSTEMS

Our knowledge on human impacts on marine ecosystems has mainly been driven by observations supported by models for extrapolation. However, there is a significant lack of data on human pressures and marine effects, particularly in the open ocean. Data are often scattered in time and space, because they mostly arise from various research cruises and ships-of-opportunity; uncoordinated activities not aimed at assessing changes over time. Therefore, models are needed to integrate these data (e.g., Boyce et al., 2010; Halpern et al., 2012), but for many components of ocean health such models do not exist or they are so coarse that the reliability of the output may be disputable (Mackas, 2010; McQuatters-Gollop et al., 2010; Rykaczewski and Dunne, 2011).

Remote sensing data from satellites overcome the problem of spatial and temporal sampling heterogeneity and can be used for assessing changes in sea surface temperature and ocean color from which proxies for phytoplankton biomass and productivity can be derived (Behrenfeld et al., 2006), but they also have their limitations. Remote sensing applies to the upper surface layer only, and satellites cannot assess processes taking place at deeper depths. Algorithms for processing remote sensing data have mainly been developed for the open ocean, and the algorithms produce biases in shallower coastal waters. The proxy information obtained from satellite imagery provides only a small fraction of information needed to assess human impact on marine ecosystems.

Autonomous sensors typically placed on fixed buoys or floatable undulating devices such as Argo floats complement remote sensing by providing subsurface information on salinity, temperature, oxygen, and bio-optical properties (Roemmich et al., 2009). For instance, Argo float data with the support of global climate models revealed that the deep ocean (>300 m) was taking up more heat during the recent surface-temperature hiatus period (Meehl et al., 2011). At present, only the most basic physical-chemical variables are measured using these autonomous devices, since other measurements of interest (e.g., nutrient concentrations) typically require more regular maintenance, increasing the operating costs substantially.

Monitoring programs providing more consistent time series across a wide range of different physical, chemical and biological variables are found in certain coastal areas, e.g., the Chesapeake Bay and the Baltic Sea. These were typically initiated in the 1970s and 1980s, when pollution effects became clearly visible, to assess the efficiency of management actions to alleviate human pressure on overstressed marine ecosystems (Carstensen et al., 2006). In addition to assessing physical-chemical status, different organism groups from phytoplankton to top predators in the marine ecosystems were monitored. These monitoring programs have contributed substantially to our present understanding of trophic interactions in coastal areas and the disturbance of these imposed by human activities.

Understanding of long-term variations in ocean waters has so far been based on a few observatories, some of these organized within the Long Term Ecological Research (LTER) Network (www.ilternet.edu). Long-term decreases in pH and aragonite saturation from the Hawaiian Ocean Time-series (HOT) and Bermuda Atlantic Time Series (BATS) have highlighted another problem associated with increased emission of CO_2, namely ocean acidification (Doney et al., 2009), which may alter ocean biogeochemistry (Beman et al., 2011). Long-term time series in coastal waters have revealed that pH is governed by changes in inputs from land rather than CO_2 in the atmosphere (Duarte et al., 2013). The Continuous Plankton Recorder (CPR) survey has been in operation since 1931 and has provided valuable insights into how climate oscillations affect plankton communities (Edwards et al., 2009). Since 1949 the California Cooperative Oceanic Fisheries Investigations (CalFOCI) program has investigated distributions of phytoplankton, zooplankton and fish distributions off Southern California and showed how changes in the Pacific Decadal Oscillation (PDO) can precipitate sudden shifts in these distributions (McGowan et al., 2003). Nevertheless, despite the value of these unique time series there is a need to establish and maintain ocean time series of high research quality, particularly in subtropical and tropical waters that are severely understudied at present.

DIRECTIONS FOR THE FUTURE

"We know more about the surface of the Moon and about Mars than we do about the deep sea floor, despite the fact that we have yet to extract a gram of food, a breath of oxygen or a drop of water from those bodies." This statement by Dr. Paul Snelgrove clearly articulates the need for improving our understanding of how marine ecosystems function, particularly as they provide essential ecosystem services to humans and because expanding human activities are putting these services under threat.

Our current understanding of marine ecosystem responses to human activities is limited by the availability of data, particularly long-term time series of physical and chemical conditions as well as biological properties. Moreover, efforts should be made to improve the accessibility and comparability of existing time series. Further development of models integrating monitoring data is needed to better assess changes over time and predict future trends, but models cannot stand alone without data. The lack of data is partly technical, as current measurement techniques may not necessarily provide the needed information, and partly financial, as costs of ocean sampling are indeed excessively expensive. Technological developments are expected to contribute more accurate, precise and cost-effective measurements over time. However, many marine monitoring programs are facing budget reductions, which have led to discontinuation of monitoring stations and abandoning sampling of biological components as well as decreasing monitoring frequencies. A possible consequence is loss of invested capital for establishing such long-term time series, simply because their value has to be written down. There is a growing discrepancy between the need for better understanding of human impact on marine ecosystems and the basis for addressing these scientific questions.

Ducklow et al. (2009) have identified seven key elements that will help science address critical issues on marine ecosystem services in times when human pressures on these are intensifying: (1) maintain existing monitoring programs and expand these with additional biological components, (2) establish new monitoring programs in under-sampled regions, (3) increase the use of remote sensing and autonomous monitoring devices, (4) establish targeted research program (process studies) in connection to long-term monitoring sites, (5) improve the integration of monitoring activities with ships-of-opportunity, (6) modify current funding for ecological research to balance consistent long-term research and short-term targeted studies, and (7) improve data access and synthesis using models. If these are recommendations are pursued we may eventually know more about our oceans than the surface of the Moon and Mars. The growing human imprint on marine ecosystems may, if left unmonitored and unattended, result in significant losses of ecosystem services that are crucial to support a globally growing population.

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Indicators to assess the status

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An Objective Framework to Test the Quality of Candidate Indicators of Good Environmental Status

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Large efforts are on-going within the EU to prepare the Marine Strategy Framework Directive's (MSFD) assessment of the environmental status of the European seas. This assessment will only be as good as the indicators chosen to monitor the 11 descriptors of good environmental status (GEnS). An objective and transparent framework to determine whether chosen indicators actually support the aims of this policy is, however, not yet in place. Such frameworks are needed to ensure that the limited resources available to this assessment optimize the likelihood of achieving GEnS within collaborating states. Here, we developed a hypothesis-based protocol to evaluate whether candidate indicators meet quality criteria explicit to the MSFD, which the assessment community aspires to. Eight quality criteria are distilled from existing initiatives, and a testing and scoring protocol for each of them is presented. We exemplify its application in three worked examples, covering indicators for three GEnS descriptors (1, 5, and 6), various habitat components (seaweeds, seagrasses, benthic macrofauna, and plankton), and assessment regions (Danish, Lithuanian, and UK waters). We argue that this framework provides a necessary, transparent and standardized structure to support the comparison of candidate indicators, and the decision-making process leading to indicator selection. Its application could help identify potential limitations in currently available candidate metrics and, in such cases, help focus the development of more adequate indicators. Use of such standardized approaches will facilitate the sharing of knowledge gained across the MSFD parties despite context-specificity across assessment regions, and support the evidence-based management of European seas.

Keywords: ecosystems, European union, good environmental status, indicator, marine strategy framework directive, pressure, water framework directive (WFD)

INTRODUCTION

The current paradigm of marine management in Europe determines that decisions should be weighed on their impacts on whole ecosystems rather than on individual ecosystem components (United Nations, 1992; MEA, 2005). This "ecosystem approach" is enshrined in the EU Marine Strategy Framework Directive (the MSFD, EC, 2008; EU, 2014) and associated Maritime Spatial Planning Directive (EU, 2014). Component parts to this approach are the aims to attain and preserve "good environmental status" in EU waters ("GEnS," EC, 2008), the definition of which has been summarized across 11 descriptors. Various initiatives have consequently proposed metrics that could serve as indicators for these descriptors to support their monitoring (hereafter "indicators," e.g., Rice et al., 2012; Borja et al., 2013), and efforts are being made to review a wealth of available and new metrics (hereafter, "candidate" indicators, or "candidate" metrics, Borja et al., 2014; Teixeira et al., 2014). As the assessment of GEnS is the fundamental aim of the MSFD, the credibility of this policy depends on the choice of adequate GEnS indicators for its descriptors. Various indicator quality criteria have since been suggested as the desirable characteristics of GEnS indicators that are fit for purpose, and discussions regarding their assessment are being undertaken (Borja et al., 2013; ICES, 2013b, 2015; Rossberg et al., 2013; Hummel et al., 2015). Additionally, scoring systems for the assessment of candidate indicators have been proposed by ICES (2013b, 2015) using a set of 16 quality criteria. However, a stringent framework for assessing whether these candidate indicators actually meet this or other sets of desired quality criteria, that is both comprehensive and applicable across the 11 descriptors of GEnS, has not been described. Though the desirable traits of a GEnS indicator may be intuitive, it is difficult to define objectively whether a candidate metric actually possesses such traits. Judgments or values thus need to be objectively laid out to enable the comparison of candidate metrics, so that an informed selection can be made across descriptors, and a smaller list of indicators ultimately suggested for implementation of the MSFD. This study aimed to provide a standardized procedure to evaluate the quality of candidate indicators across the descriptors, through objective analysis and testing. This framework lays out a transparent and repeatable methodology to test the fulfillment of quality criteria that can be used to define indicator quality, and to rank candidate indicators to facilitate indicator selection within the MSFD assessment.

From a wide range of published alternatives (**Table 1**, adapted from Krause-Jensen et al., 2015) the ICES quality criteria for selecting MSFD GEnS indicators for the North Sea (ICES, 2013a,b, 2015) were chosen as a basis for the present study because this list already resulted from previous exercises to synthesize published efforts, reflecting common aspirations within the community. This ICES quality criteria list has already been applied for selecting common OSPAR (the Convention for the Protection of the Marine Environment of the North-East Atlantic) indicators for the MSFD (ICES, 2015). The list describes 16 quality criteria which were here further distilled to eight Indicator Quality criteria [henceforth, "IQ(s)," **Figure 1** and Table S1]. This simplification was deemed necessary to facilitate the operationalization of indicators by reducing perceived overlap within that list and keeping the focus on state indicators and key performance criteria for these (Table S1 for justification, from Krause-Jensen et al., 2015). Based on these eight IQs, a framework for the analysis of candidate GEnS indicators is presented here which: (1) formulates objective, transparent and repeatable tests of indicator quality; (2) constructs a ranking system to enable the comparison of alternative candidate indicators and thus facilitate indicator selection; and (3) quantitatively displays indicator strengths and weaknesses, and hence the potential need for additional indicator development. Within a wide range of available candidate metrics, four falling within the remit of expertise of the authors, were chosen to investigate and demonstrate the application of this framework as worked examples.

MATERIALS AND METHODS

The proposed indicator quality testing framework is detailed below, followed by three worked examples detailing its application to four candidate metrics. These metrics currently exist at different stages of operationalization as candidate indicators for the MSFD. Presentation of these worked examples was thus not primarily aimed to serve as actual tests of their quality as actual indicators for the MSFD (although this text could potentially come to support that aim). Rather, they are detailed here with the specific aims of investigating and demonstrating the application of the proposed testing framework across a variety of GEnS descriptors, indicator and ecosystem types, to help build the case for, and support, its further uses by the community. Specifically: the quality of presence of keystone kelp species and the depth limit of eelgrass as candidate metrics for descriptors 1 (biodiversity) and 5 (eutrophication) in the Danish coast is evaluated in worked example I; the quality of the temporal trend of N:P in coastal waters as a potential indicator for the occurrence of harmful algal blooms under descriptor 5 (eutrophication) in the UK is evaluated in worked example II; and the quality of the Benthic Quality Index (BQI, Fleischer et al., 2007) as a potential indicator for descriptors 1 (biodiversity) and 6 (seafloor integrity) in the Lithuanian coast is evaluated in worked example III. With regard to their current status of operationalization: seagrass depth limits are already considered in Denmark and other European countries as indicators for ecological status under the Water Framework Directive ("WFD"), and are being considered within the MSFD (Marbà et al., 2013); presence of kelps is being considered by ICES and specific European countries as a potential indicator for descriptor 1 of the MSFD, though not yet in Denmark (Burrows et al., 2014; Hummel et al., 2015); the trend of N:P is not yet being considered by the MSFD, although the data required for its estimation is collected routinely as part of WFD monitoring efforts around Europe; the BQI is already extensively in use by Baltic countries to assess ecological status for the WFD, including by the Lithuanian Environment Ministry (Šiaulys et al., 2011), and it is under consideration for the

TABLE 1 | Literature survey of the use of indicator quality criteria (IQ).

Indicator Criteria identified in literature survey	ICES, 2013a^	ICES, 2013b^	ICES, 2015^	OSPAR, 2013	HELCOM, 2012	JCN/HBDSEG, 2012	Rice, 2003	Borja et al., 2004	Rice and Rochet, 2005	Mee et al., 2008	Niemeijer and de Groot, 2008^^	Elliott, 2011	Mazik et al., 2010	Kershner et al., 2011; James et al., 2012^^^	TOTAL USE
IQ1: SCIENTIFIC BASIS	1	1	1		1	1	1		1	1	1	1	1	1	**12**
IQ2: ECOSYSTEM RELEVANCE*	1	1	1	1			1			1	1	1		1	**9**
IQ3: RESPONSIVENESS TO PRESSURE	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**14**
- Responsive, Sensitive, Specific, Predictable**	1	1	1	1	1	1	1	1	1		1	1	1	1	13
- Time-scale of response	1	1	1			1	1	1		1	1	1			9
IQ4: POSSSIBILITY TO SET TARGETS	1	1	1				1				1	1		1	**7**
IQ5: PRECAUTIONARY CAPACITY/EARLY-WARNING/ANTICIPATORY	1	1	1			1				1	1	1			**7**
IQ6: QUALITY OF SAMPLING METHOD (concrete, measurable, accurate, precise, and repeatable)	1	1	1	1	1	1	1		1	1	1	1	1	1	**13**
- Concrete	1	1	1						1		1	1		1	7
- Quantitative/measurable***	1	1	1	1	1	1				1	1	1		1	10
- Accurate/precise/robust****	1	1	1		1	1	1		1		1		1	1	10
IQ7: COST-EFFECTIVE	1	1	1			1	1		1	1	1	1		1	**10**
IQ8: EXISTING AND ONGOING MONITORING DATA	1	1	1		1	1	1	1	1		1			1	**10**
- Existing and ongoing monitoring data	1	1	1		1		1	1			1			1	8
- Historical data						1	1		1		1			1	5
CRITERIA WE CONSIDER IMPLICIT IN IQ1–IQ8															
- Meaningful/understandable—implicit in IQ1–3	1	1	1			1	1				1		1	1	8
- Legal/policy relevance—implicit in IQ1–3					1	1	1				1				4
- Social relevance—implicit in IQ2									1	1	1	1			4
- Management linkage—implicit in IQ1–3	1	1	1	1	1	1	1	1			1	1	1	1	12
SECONDARY QUALITY CRITERIA															
- Coupling with other indicators/indicator suites*****	1	1	1		1	1						1		1	7
- Non-destructive												1			1
- Simple/easy	1	1	1			1					1		1	1	7
NOT CONSIDERED IN OUR INDICATOR TEST															
- Large spatial coverage/portability	1	1	1	1	1	1		1		1	1	1	1	1	12
- Established/commonly agreed/international******	1	1	1	1	1			1			1			1	8
- Harmonized methodology					1									1	2

In the present study, we incorporated the eight criteria marked in bold, IQ1–IQ8. A number of additional criteria were considered implicit in IQ1–IQ8, some criteria were considered of secondary importance, and some criteria (regarding large-scale applicability and commonly accepted status) were excluded from our framework on the basis that indicators fulfilling the key criteria IQ1–IQ8 are also potentially relevant for large-scale application and acceptance. Adapted from Krause-Jensen et al. (2015). Further justification for distilling the 16 ICES criteria to 8 key criteria are provided in Table S1.

^The ICES (2013a,b); ICES (2015) criterion "state or pressure indicator" is not included here as our test is focused on state indicators.

^^Based on Schomaker (1997), OECD (2001), NRC (2000), Dale and Beyeler (2001), CBD (1999), Pannell and Glenn (2000), Kurtz et al. (2001), EEA (2005).

^^^Based on a total of nineteen evaluation criteria gleaned from the literature (O'Connor and Dewling, 1986; Landres et al., 1988; Noss, 1990; Harwell et al., 1999; Jackson et al., 2000; Kurtz et al., 2001; Rice, 2003; Jennings, 2005; Rice and Rochet, 2005; Niemeijer and de Groot, 2008; Doren et al., 2009; Jørgensen et al., 2010).

**Includes also: metrics should fit indicator function (ICES criterion #14); biologically important (Elliott, 2011), representable (OSPAR), integrative and general importance (Niemeijer and de Groot, 2008).*

***Includes also: space-bound (sensitive to changes in space, Niemeijer and de Groot, 2008).*

****Includes also: practicable.*

*****Includes also: confidence evaluation; uncertainty about level (Niemeijer and de Groot, 2008), and "limitations defined" JCN/HBDSEG (2012).*

******Includes also: suitability w. assessment tools (HELCOM, 2012).*

*******Includes also: reliability (Niemeijer and de Groot, 2008).*

Aim: Objective, Transparent and Repeatable Assessment of Quality of Candidate Indicators	CANDIDATE INDICATORS
INDICATOR QUALITY CRITERIA (IQ)	**EVALUATION STEPS (ES)**
IQ1. Scientific basis	ES1 – ES5
IQ2. Ecosystem relevance	ES1 – ES5
IQ3. Responsiveness to pressure	ES1 – ES5
IQ4. Possibility to set targets within the indicator response	ES1 – ES5
IQ5. Precautionary capacity/early warning/anticipatory capability	ES1 – ES5
IQ6. Quality of sampling method: measurable, accurate and precise outputs	ES1 – ES5
IQ7. Cost-effective implementation	ES1 – ES5
IQ8. Part of an existing or current ongoing monitoring or data	ES1 – ES5
ES6. Sum of quality scores across IQs, per indicator	$\sum_{i=1}^{8} IQ_i$ (ES5)
Comparison of ES6 Scores For Candidate Indicators	SELECTION OF HIGHEST SCORING INDICATOR

FIGURE 1 | Overview of the elements in the IQ-ES framework for candidate indicator selection. Candidate indicators are tested on the basis of eight indicator quality criteria (IQ1–IQ8), each of which are evaluated and scored through five sequential steps (ES1–ES5). The final score for each candidate indicator is calculated across IQ1–IQ8 in evaluation step 6 (ES6). The comparison of the total quality score of candidate indicators is intended to provide an objective and transparent basis to inform indicator selection.

MSFD; it is already being implemented in Sweden under this directive.

Quality Testing: The IQ-ES Framework

The indicator evaluation framework is detailed in the next section. For a given candidate indicator (A) or a pair of candidate indicators (A and B) of the same descriptor of GEnS being compared, a sequence of five Evaluation Steps (henceforth "ES") was defined for each of eight IQs to determine whether each is met (**Figure 1**). In summary, ES1 states the null hypothesis associated with the IQ tested; ES2 defines which assessment approach should be employed to test the hypothesis, i.e., qualitative or quantitative, and is conditional to its nature; ES3 states the type of evidence required to undertake the assessment; ES4 defines the methodology (e.g., type of statistical analysis or otherwise) undertaken to test the hypothesis considered and its outcome; ES5 states the quality score for the particular IQ tested given ES4. If the test is successful (within the assessment of each of the eight IQs), the indicator scores 1 in the final step (**Figure 1**, ES5) and 0 otherwise. Once IQs 1–8 have been assessed through these steps individually, all scores are summed in a final step (**Figure 1**, ES6) and a total quality score for the candidate indicator is calculated, which can be compared to that of other candidate indicators for the same descriptor.

At the core of this assessment structure is the expression of each IQ into a testable null hypothesis (ES1). In keeping with a statistical testing background, the hypothesis is stated as a negative that is rejected if the indicator meets the IQ tested for, and accepted otherwise (ES5). Without this first step, there is no clarity about what attribute of quality is being assessed. For example, in IQ1 (**Figure 1**, "scientific basis") ES1 (the null hypothesis) states that "there is no scientific basis for the indicator." Based on the review of associated literature, an informed judgment can be made: the analysis that tests this hypothesis is therefore qualitative and the outcome is categorical (yes or no). Examples of qualitative approaches may therefore include expert judgment, by which e.g., a review of literature may be sufficient to establish whether the indicator satisfies a particular criterion of quality. Conversely, in IQ3 (**Figure 1**, "responsiveness to pressure"), ES1 is only truly testable under a quantitative approach, requiring that a minimum pressure change of interest induces a measureable and consistent indicator response, for the system analyzed. Quantitative approaches could include statistical analyses, graphical exploration of data, or any type of numerical modeling to define a quantitative relationship. The nature of the hypothesis defined by ES1 therefore dictates which type of approach should be preferred in ES2 (qualitative c.f. quantitative). The preferred type of approach (**Figure 1**, ES2) in turn helps identify which type of evidence, resources (**Figure 1**, ES3), and analyses (**Figure 1**, ES4) need to be considered for the assessment of each specific IQ, for each indicator and context (i.e., descriptor, area).

Whilst the analysis method used in ES4 may be substantially different between candidate metric types, the comparison of metrics to enable selection requires that the quality assessment is standardized across these metrics within descriptors. We suggest that this quality scoring system provides this comparative basis. Various weighted and non-weighted scoring systems are possible in ES5. However, given that the key aims of this framework are the objective, transparent and repeatable evaluation and ranking of indicators according to quality criteria, we suggest that the use of a binary system (0,1) provides the most unambiguous statement of the assessment outcome: that the indicator does (1) or does not (0) meet the quality criterion tested. However, here, we compare this approach with that suggested by ICES (2013a,b, 2015), which includes an additional possible score (0.5) in IQs 2 and 4–8, expressing that a given quality criterion is partially fulfilled (three-way scoring system).

We suggest that once ES1–6 have been undertaken for a pair of candidate indicators (e.g., A and B) for a given descriptor, their total quality score (ES6) should provide a sufficient basis for a pair-wise comparison and selection, with preference given to the indicator with the highest score. This is a fundamental step toward an objective sorting and selection of candidate indicators, ensuring consistency, comparability, transparency and repeatability of the selection approach regardless of the indicator, descriptor, pressure, habitat, or biological component assessed. Overall, this general framework thus converts aspirational attributes (Table S1) associated with the definition of indicators into a series of defined, analytical steps to establish GEnS candidate indicator quality. IQ1 and IQ3 are seen as essential quality criteria in the assessment, such that failure to meet either of these criteria should render exclusion. In other words, IQ1 and IQ3 are "one-out-all-out" criteria. Overall score ties between candidate indicators (ES6) compared using this framework require expert judgment for selection (see also Table S1). Here too, the standardized format of the IQ-ES assessment could set a good basis to inform this decision because the quality assessment is broken down into its component criteria.

The GEnS Indicator Quality Evaluation Steps

IQ 1: Scientific basis (one-out-all-out criterion)

IQ1–ES1: there is no scientific basis for the indicator.
IQ1–ES2: expert judgment/qualitative approach are adequate.
IQ1–ES3: publications evidencing the conceptual basis for using the indicator, stressing the existence of a general causal link between the indicator and a given pressure, highlighting an effect on the relevant descriptor. Peer-reviewed publications are preferred but, in some instances, reports from governmental institutes or international institutions (e.g., ICES) may be more appropriate.
IQ1–ES4: the indicator must be reproducible, i.e., the conceptual basis and causality relationship have been published (preferentially in peer-reviewed literature) using multiple data sets, and this can be seen as a proxy for its wide acceptance within the relevant scientific community.
IQ1–ES5: the indicator scores 1 if the above can be verified. If the indicator scores 0 in IQ1, it is seen as failing in the quality assessment as this is a one-out-all-out quality criterion. Because of this, we consider that the three-way scoring system is not applicable to IQ1.

IQ 2: Ecosystem relevance

IQ2–ES1: there is no evidence linking the indicator to (a) ecosystem level processes and function (the non-anthropocentric perspective; e.g., indicators of processes undertaken by keystone species could be particularly relevant); and/or (b) ecosystem services (the anthropocentric perspective, i.e., societal relevance).
IQ2–ES2: expert judgment/qualitative approach are adequate.
IQ2–ES3: scientific, peer-reviewed evidence for the non-anthropocentric criterion and/or for the anthropocentric criterion.
IQ2–ES4: a literature review is a recommended approach to test IQ2. Evidence for the ecosystem relevance of the indicator should have been published in peer-reviewed literature. Within the anthropocentric perspective, the indicator must be explicitly listed within recognized ecosystem function/service typologies, or they have been linked directly to a monetary valuation. For instance, indicators listed under the Common International Classification of Ecosystem Services (Haines-Young and Potschin, 2013) or another equally widely applied typology are preferred.
IQ2–ES5: the indicator scores 1 if the above (IQ2–ES4) can be verified and 0 otherwise. The three-way scoring system could be applied to IQ2.

IQ 3: Responsiveness to pressure (one-out-all-out criterion)

IQ3–ES1: the indicator does not exhibit consistent and significant change as a result of a change in pressure, as listed within the recognized MSFD pressure list (EC, 2008), in the system of interest.
IQ3–ES2: a quantitative approach is adequate.
IQ3–ES3: the data used for testing should include some information about the natural baseline of the system, including information about its natural variability because this may confound the ability to detect a pressure driven effect. The drivers of the natural variability baseline of the indicator are known and understood. In case data for the area in question is not sufficiently comprehensive to allow proper pressure-response analyses, pressure-response analyses conducted for the same candidate indicator in comparable ecosystem(s) could be considered.
IQ3–ES4: the method of analysis must consider the impact/influence of natural variability (if any) on the response of the indicator (identify, estimate, and diagnose). The analysis must be appropriate for the complexity of the data to hand.
IQ3–ES5: the indicator scores 1 if a consistent and significant change is measured in response to the pressure (IQ3–ES4), and 0 if: (i) there is no change in response to pressure; or (ii) the change in the indicator in response to pressure is not consistent (across areas, scales); or (iii) the measured change in the indicator in response to the pressure is not statistically significant. If the indicator scores 0 in IQ3, it is seen as failing in the quality assessment as this is a one-out-all-out quality criterion. Because of this, the three-way scoring system is not applicable to IQ3.

IQ 4: Possibility to set targets

IQ4–ES1: a clear and unambiguous target cannot be defined for the indicator within a range with defined units of measurement.
IQ4–ES2: both expert judgment/qualitative approach and a quantitative approach can be adequate, depending on the indicator.
IQ4–ES3: information about the range of natural variability of the system is required, against which the target level is defined.
IQ4–ES4: the method of analysis must consider the impact/influence of natural variability (if any) on the response of the indicator (identify, estimate, and diagnose). The analysis must be appropriate for the type of data at hand (qualitative c.f. quantitative).
IQ4–ES5: the indicator scores 1 if a clear and unambiguous target can be defined with clear units of measurement, and 0 if: (i) a clear and unambiguous target cannot be defined; or (ii) there is not sufficient background information to define the range of the natural variability of the system (i.e., habitat and scale) within which the indicator is to be implemented. The three-way scoring system could be applied to IQ4.

IQ 5: Precautionary capacity/early-warning/anticipatory

IQ5–ES1: there is no immediate and measurable change in the indicator associated with a change in the pressure that anticipates ecosystem-level change in the system (see IQ2).
IQ5–ES2: a quantitative approach is adequate.
IQ5–ES3: data that enables a quantification to be made about the time lag between pressure level and indicator response, and that between pressure change and ecosystem-level relevant change. Information must exist about a clear link between pressure level and ecosystem state. The indicator must be responsive to pressure (IQ3). These data are particularly important in instances where system collapse may occur. The rate of change in the indicator during impact and recovery phases may be distinct.
IQ5–ES4: any quantitative method of analysis that measures the lag time between pressure and indicator response, and the lag between pressure change and ecosystem-level change. The indicator analysis method must be reproducible (IQ6).
IQ5–ES5: the indicator scores 1 if the lag time between pressure change and the detection of a measurable change in the indicator level is small and suitable to enable mitigation action to take place to prevent ecosystem-level change. The indicator scores 0 if the time lag between pressure change and indicator response is not sufficiently small to support action taking place within the system to prevent further ecosystem scale deterioration. The three-way scoring system could be applied to IQ5.

IQ 6: Quality of sampling method: Concrete/measurable, accurate, precise and repeatable

IQ6–ES1: the indicator is not concrete/measurable, accurate, precise or repeatable. Concreteness/measurability refers to whether the indicator can be quantitatively assessed. Accuracy refers to the closeness of an estimate of an indicator to the true value of the indicator. Precision refers to the degree of concordance among a number of estimates for the same population and repeatability to the degree of concordance among estimates obtained by different observers (Sokal and Rohlf, 1969).
IQ6–ES2: a quantitative approach is adequate.
IQ6–ES3: identification of whether an indicator is concrete/measurable requires availability of well-defined quantitative data. Testing for accuracy requires quantitative data to address the possibility of measurement bias. Testing for precision requires data covering spatial and temporal scales of variability and is necessary for quantifying how much sampling effort is required to identify an effect size of a defined level in the indicator in the context of the spatial- and temporal variability of the system being assessed. Testing for repeatability requires data allowing comparability of estimates obtained by two or more different observers.
IQ6–ES4: For the analysis of concreteness/measurability, any method that enables well-defined quantitative

information on the indicator can be used. For testing accuracy and precision and repeatability, analyses of variability are suitable and these can be supplemented with power analysis and species area curves to evaluate the necessary sampling effort.

IQ6–ES5: the indicator scores 1 only in the case in which all analyses in IQ6–ES4 lead to the rejection of the null hypothesis set out by IQ6–ES1. The indicator scores 0 if the hypothesis cannot be rejected for one or more of the attributes (i.e., if the indicator cannot be positively identified as being simultaneously concrete, accurate, precise, and repeatable). In the case of score ties, indicators for which the most attributes in IQ6 could be validated are preferred. The three-way scoring system could be applied to IQ6.

IQ 7: Cost-effective

IQ7–ES1: the indicator is not cost effective.

IQ7–ES2: a quantitative approach is adequate.

IQ7–ES3: requires information about the levels of precision and accuracy required (IQ6), against which the costs of the necessary method of implementation of the indicator are calculated.

IQ7–ES4: any analysis that enables the establishment of the change in cost associated with an improvement in the criteria of accuracy and precision of the indicator.

IQ7–ES5: the indicator scores 1 if the cost associated with the desired level of precision and accuracy is manageable and 0 otherwise. The three-way scoring system could be applied to IQ7.

IQ 8: Existing and ongoing monitoring data

IQ8–ES1: the indicator is not currently used in ongoing monitoring program(s).

IQ8–ES2: a quantitative approach is adequate.

IQ8–ES3: requires information about the length of time during which the indicator has been in use within a monitoring program, and of the redundancy the indicator in relation others (if any) also in use within the scale of analysis of interest.

IQ8–ES4: any method that quantifies the above (IQ8–ES3).

IQ8–ES5: the indicator scores 1 if is already in use in at least one monitoring program, and 0 otherwise. In a score tie, indicators with the longest use of application, exhibiting potential for application in the widest areas of interest, are preferred. The three-way scoring system could be applied to IQ8.

ES6 sum of quality scores

The scores given in ES5 in IQ1–8 are summed, ranging between 0 and 8.

Worked Examples

We exemplify the application of this framework in three case-studies, assessing potential candidate indicators of marine

TABLE 2 | Datasets used in worked examples of the application of the IQ-ES framework for quality testing and selection of GEnS indicators.

Worked example	Dataset	MSFD pressure	Location	Depth (m)	Position	Time frame	Research type	Data Owner	Data contact
Benthic vegetation	Kelp and seagrass diversity; water depth; nutrient concentrations; light characteristics.	Nutrient loading	Danish Coast	0–100	N 56° 09.93′ E 10° 13.25′	1989–present	from DNAMAP (Danish National Aquatic Monitoring and Assessment Program)	Danish Nature Agency	Dorte Krause-Jensen and Jacob Carstensen
Benthic macrofauna	Taxonomic composition, abundance, biomass of macrofauna; seawater temperature, salinity, phosphate concentration, phosphorus concentration, nitrate concentration, nitrogen concentrations, chlorophyll a concentration.	Nutrient loading	Lithuanian shallow coastal waters	13–20	N 56°03.75′ E 21°02.00′	1981–2012	Commissioned research by Klaipėda University and Lithuanian Environment Protection Agency, Marine Research Department	Klaipėda University (D. Daunys) and Lithuanian Environment Protection Agency	Romualda Chuševė
Phytoplankton	Nitrate and phosphate concentrations; phytoplankton diversity and abundance.	Nutrient loading	South West of the United Kingdom	50	N 50°15.00′ W 4° 13.02′	2000–2013	Western Chanel Observatory	Plymouth Marine Laboratory	Stefano Ciavatta

ecosystem components ranging from nutrients and benthic vegetation to soft sediment faunal communities (**Table 2**). For practical reasons, we provide only one worked example in the main body of the text, analyzing two candidate indicators; two other worked examples are explored in the same level of detail in the Supplementary Materials Section.

RESULTS

Worked Example I. Candidate Indicators for Descriptors 1 (Biodiversity) and 5 (Eutrophication): Presence of Keystone Kelp Species and Eelgrass Depth Limit

In this example, we comparatively evaluate the quality of two candidate indicators which could be used to monitor both descriptor 1 (Biodiversity) and descriptor 5 (Eutrophication), within Danish waters. Specifically, we compare the quality of: the presence of keystone kelp species (seaweeds) and the depth limit for eelgrass (a seagrass). This evaluation is summarized in **Table 3**.

IQ 1. Scientific Basis

Both candidate indicators and their general responses to human driven nutrient loading pressure (causing eutrophication) are conceptually well founded in the scientific literature. More specifically, Duarte (1991) and Duarte et al. (2007) demonstrated a global trend that deeper seagrass meadows occur in clearer waters. This relationship is supported by studies in Danish coastal waters, where the depth limit of eelgrass is largest in the clearest waters with lowest nutrient concentrations (Nielsen et al., 2002; Greve and Krause-Jensen, 2005; Krause-Jensen et al., 2011). Markedly deeper meadows than those found at present were found during past periods of lower nutrient inputs (Boström et al., 2014). Similarly, spatio-temporal data from Norway's coast indicate declines in kelp forests in response to nutrient loading causing eutrophication (Moy and Christie, 2012). Therefore, literature exists that has linked both of these candidate indicators to eutrophication, which is listed by the MSFD as reflecting poor GEnS (descriptor 5). In addition, kelp forests and seagrass meadows constitute habitat for a vast diversity of species (Gutiérrez et al., 2011; Boström et al., 2014). Therefore, both indicators are also linked to the descriptor 1 (Biodiversity). Both candidate indicators therefore scored 1 in IQ1 (**Table 3**).

IQ 2. Ecosystem Relevance

Kelp forests and seagrass meadows are so-called keystone species and ecosystem engineers, providing a whole range of additional ecosystem functions and services including coastal protection, seafloor stabilization, carbon and nutrient retention, and promotion of water clarity (Costanza et al., 1997; Gutiérrez et al., 2011; Duarte et al., 2013). Both candidate indicators therefore scored 1 for IQ2 (for both descriptors), fulfilling the criterion of ecosystem relevance, from both anthropocentric and non-anthropocentric perspectives.

IQ 3. Responsiveness to Pressure

The trend of deeper seagrass meadows in clearer and less nutrient-rich waters has been demonstrated in the case-study system (Danish waters, Nielsen et al., 2002; Greve and Krause-Jensen, 2005; Krause-Jensen et al., 2011; Riemann et al., 2016) and globally (Duarte et al., 2007). It is, however, important to note that while response to increased nutrient pressure may be quick, the recovery of this vegetation following reduced nutrient inputs may require long time frames (Krause-Jensen et al., 2012; Duarte et al., 2015; Riemann et al., 2016). Hence, eelgrass depth limits have been found to exhibit no signs of improvement after 15 years of nutrient input reductions in a shallow German bay (Munkes, 2005) while in Danish coastal waters, recovery has been observed more than 2 decades after nutrient input reductions (Hansen, 2013; Riemann et al., 2016). Several sources of variability have been tested for eelgrass depth limits (a requirement to meet this IQ in the present framework), the most important being spatial variability, which must be carefully addressed in the planning of monitoring programs (Balsby et al., 2013). Hence, with respect to the responsiveness criterion, eelgrass depth limits scored 1 in IQ3.

With respect to the presence of kelps, spatio-temporal data from Norway's coast indicate declines in kelp forests in response to nutrient loading (and warming) causing eutrophication (Moy and Christie, 2012). By contrast, a recent Danish study showed no response of the presence of kelps to varying nutrient concentrations (Krause-Jensen et al., 2015) indicating that this candidate indicator is not sufficiently sensitive near the geographical distribution limit, where low salinity and high summer temperatures constrain growth (Nielsen et al., 2014). Kelp presence scored 0 in the binary scoring system. As this is one of the most important quality criteria (i.e., one of the two "one-out-all-out" criteria), the presence of kelps as indicators for descriptor 1 (and 5) of GEnS would be rejected under the current assessment framework.

IQ 4. Possibility to Set Targets

Historical information on eelgrass depth limits from a period with limited nutrient input can form a suitable basis for establishing targets for eelgrass depth extension in Danish coastal waters, and pressure-response relationships can also be used for target-setting (e.g., Carstensen and Krause-Jensen, 2009) whilst considering the natural variability of this candidate indicator. Conversely, no clear pressure-response relationship between presence of even the most common kelps in the area [*Saccharina latissima* (Linnaeus) and *Laminaria digitata* (Hudson)] and nutrient pressure can be established at present to support target setting for this candidate indicator. Therefore, seagrass depth scored 1 in IQ4, whilst keystone kelp presence scored 0 in this particular example. Targets for both species should always be identified for the particular areas of interest.

IQ 5. Precautionary Capacity/Early-Warning/Anticipatory

The early warning capacity of both candidate indicators assessed is limited. Eelgrass depth limits scored 0 on this criterion

TABLE 3 | Summary of quality assessment of the candidate benthic vegetation indicators "Presence of keystone kelp species" and "Eelgrass depth limit," both relating to the MSFD indicator category "Distributional pattern (1.4.2), in association with the GEnS descriptors 1 and 5.

Quality criterion	Evaluation Step	Eelgrass depth limit	Presence of keystone kelps
IQ1: Scientific Basis	ES1	There is no scientific basis for the indicator.	
	ES2	Qualitative approach	Qualitative approach
	ES3	Literature review	Literature review
	ES4	Causal link to nutrient loading, and methods described: Cloern, 2001; Krause-Jensen et al., 2011	Causal link to nutrient loading, and methods described: Sand-Jensen and Borum, 1991; Schramm, 1999; Moy and Christie, 2012
	ES5	1	1
	ES1	There is no evidence linking the indicator to ecosystem-level processes or services.	
IQ2: Ecosystem Relevance	ES2	Qualitative approach	Qualitative approach
	ES3	Literature review	Literature review
	ES4	Anthropocentric and non-antropocentric criteria: Costanza et al., 1997; Gutiérrez et al., 2011; Duarte et al., 2013	Anthropocentric and non-antropocentric criteria: Costanza et al., 1997; Gutiérrez et al., 2011
	ES5	1	1
	ES1	The indicator does not exhibit consistent and significant response to the pressure.	
IQ3: Responsiveness to pressure	ES2	Quantitative approach	Quantitative approach
	ES3	Quantitative analysis of time-series data or spatial data sets	Quantitative analysis of time-series data or spatial data sets
	ES4	Spatial-temporal analysis: Nielsen et al., 2002. Time-series using GLM: Krause-Jensen et al., 2011; Riemann et al., 2016. Natural vairability: Balsby et al., 2013	Spatial-temporal analysis: Moy and Christie, 2012. Time-series using GLM: Krause-Jensen et al., 2015.
	ES5	1	0
	ES1	A clear and unambiguous target cannot be defined.	
IQ4: Possibility to set targets	ES2	Quantitative approach	Qualitative approach
	ES3	Analysis of historical data (pressure/response) including system variability	Analysis of pressure/response data
	ES4	Carstensen and Krause-Jensen, 2009	Krause-Jensen et al., 2015
	ES5	1	0
	ES1	Change in the indicator does not anticipate ecosystem-level change.	
IQ5: Precautionary capacity/early-warning/anticipatory	ES2	Quantitative approach	Quantitative approach
	ES3	Quantitative analysis of time-series data	Quantitative analysis of time-series data
	ES4	Slow response to pressure: Riemann et al., 2016	Potential for response within 1 year (Moy and Christie, 2012), but response is ambiguous (Krause-Jensen et al., 2015)
	ES5	0	1 (0.5)
	ES1	The indicator is not concrete/measurable, accurate, precise or repeatable.	
IQ6: Concrete, measurable, accurate, precise and repeatable	ES2	Quantitative approach for all qualities; qualitative for repeatability	Quantitative approach for all qualities; qualitative for repeatability
	ES3	(1) Data that allows analysis of: uncertainty in response to pressure and natural variability (concreate, measurable, accurate and precise analysis). (2) Repeatability assessed via analysis of data from multiple systems.	Large monitoring data sets to assess that the indicator is concreate, measurable accurate, precise and repeatable
	ES4	(1) Krause-Jensen and Carstensen, 2012. (2) Balsby et al., 2013	Krause-Jensen et al., 2015
	ES5	1	1
	ES1	The indicator is not cost effective.	

(Continued)

TABLE 3 | Continued

Quality criterion	Evaluation Step	Eelgrass depth limit	Presence of keystone kelps
IQ7: Cost-effective	ES2	Quantitative approach	Quantitative approach
	ES3	Assessment of the cost of the data acquisition method (underwater video/diver survey) used in relation to the other IQ	Assessment of the cost of the data acquisition method (underwater video/diver survey) used in relation to the other IQ
	ES4	Cost-efficiency can be optimized through design (Balsby et al., 2013).	Non-consensual pressure-response relationship (IQ3) deems the cost of data acquisition too high.
	ES5	1/0.5	0
	ES1	The indicator is not yet used in monitoring programmes.	
IQ8: Existing and ongoing monitoring data	ES2	Quantitative approach	Quantitative approach
	ES3	Information about the length of time during which the indicator has been in use within a monitoring program	Information about the length of time during which the indicator has been in use within a monitoring program
	ES4	Monitoring data available since 1989 and ongoing in Danish waters	Monitoring data available since 1989 and ongoing in Danish waters
	ES5	1	1
	ES6	7/6.5	5/4.5

ES1 is summarized in the text.

because of the slow response to nutrient input reduction as that recorded in Danish coastal waters (Riemann et al., 2016). This likely reflects a slow recovery of light conditions and general environmental conditions including sediment quality, suggesting feed-back mechanisms of the degraded ecosystem in play that maintain a degraded state (e.g., van der Heide et al., 2011; Duarte et al., 2015; Riemann et al., 2016). Kelps are relatively long-lived and have complex life cycles. However, there are examples from Skagerrak of disappearance as well as of recovery of *S. latissima* stands within 1 year (Moy and Christie, 2012). Therefore, the presence of kelps are scored higher than eelgrass depth limit in IQ5: 1 in the binary system and for *S. latissima* in this particular example; or 0.5 if in the three level system (ICES, 2013a), because the re-colonization potential depends on distance from source populations. Eelgrass depth limit is scored 0.

IQ 6. Quality of Sampling Method: Concrete/Measurable, Accurate, Precise, and Repeatable

Both candidate indicators are concrete/measurable and repeatable. The actual measurement methods involved in the quantifications of the candidate indicators rely solely on adequately measuring depth of seagrass meadows in one case, and identifying kelp species in the other. Both approaches are common enough in the scientific community that IQ6 should be met. Precision in the identification of response to pressure (a requirement defined for this IQ in the present framework) requires addressing factors contributing to the variability in the estimates. Several sources of variability have been tested for eelgrass depth limits, the most important being spatial variability which must be carefully addressed in the planning of monitoring programs (Balsby et al., 2013). As mentioned above, for kelp forest, the factors associated with variability are particularly relevant at the edge of their geographical distributions and this should be considered in any assessment. Given this analysis, both indicators are scored 1 in IQ6.

IQ 7. Cost-Effective

Both candidate indicators can be monitored either by diving or by the use of under-water video surveys, the latter speeding up the assessments and, in themselves, serving as documentation for the assessment. The design of monitoring programs can be optimized by combining information on sources of variability and cost assessments, as has been exemplified for eelgrass depth limits (Balsby et al., 2013). Video surveys could be preferred to diver-based surveys, because of the lowering cost of good quality imaging technologies. However, specialized operators are still required to identify the presence of seagrass species, and the acquisition of general habitat information. The presence of kelp is assigned a 0 score in IQ7 because the required effort to acquire data is seen as being too high given the context dependence of pressure-response relationships (see IQ3). Eelgrass depth limits are assigned a score of 1 in the binary system, and 0.5 in the three-way scoring system (ICES, 2013a), because responsiveness to pressure is good but the cost associated with data acquisition is still relatively high.

IQ8. Existing and Ongoing Monitoring Data

Data on both candidate indicators have been collected continuously since 1989 as part of the Danish National Aquatic Monitoring and Assessment Programme (DNAMAP) and regional monitoring activities. Therefore, both candidate indicators scored 1 in IQ8.

ES6. Sum of Quality Scores

Overall, eelgrass depth limit scored 7, and presence of keystone kelp scored 5 in the binary system. The corresponding scores were 6.5 and 4.5 in the three-way scoring system (**Table 3**). This quality analysis indicates that eelgrass depth limits is the preferable of the two candidate indicators for descriptors 1 and 5 (**Table 3**) responding to nutrient pressure in this case-study area. This results from a better pressure-response relationship and possibilities for target setting for eelgrass depth limits, although the presence of keystone kelps may potentially have better capacity as indicator of system recovery under these descriptors.

DISCUSSION

The worked examples (Section Results and Supplementary Information) demonstrate the application of the proposed quality assessment framework for distinct types of candidate indicators and separate descriptors of GEnS. Despite these differences, the application of the framework was possible, and the worked examples are expected to provide guidance in future uses of this tool by highlighting the types of data sought, and how the evaluation steps should work. The structure of the quality assessment is particularly clear in tabular form (**Table 3**, and Tables S2, S3). The joint use of this format in support of the narrative form for reporting of the quality assessment is therefore recommended, because the former enables a quick and objective overview of the assessment process while detail is provided in the latter. This is seen as being particularly useful in the comparison of the quality of candidate indicators for the same descriptor within a region. In these cases, higher quality scoring is preferable because higher scoring within compared candidate indicators highlights which metric meets the MSFD assessment aims more closely.

However, implementation of the highest scoring candidate metric locally may not always be the preferred choice against, for instance, an overall aim to produce a standardized assessment across the MSFD participating parties. Specifically, it is likely that the quality score of individual metrics will vary between countries (and regions) given regional differences in data availability, skill set, costs, and resources available for data collection and analysis, among other constrains. Therefore, this testing framework would best support the decision making process, and indicator selection, if the approach was applied to candidate metrics at least at the country level, and ideally at sub-assessment region level. In this way, it could support a standardized indicator selection process through the determination of which specific candidate metrics score the highest across participating parties for each given descriptor. The clear representation of this quality assessment provides a consistent and objective structure to inform about what desired quality attributes each candidate indicator does or does not meet in each case, and the potential need for specific development in each case. A standardized format for the assessment table could be implemented to facilitate the application of the IQ-ES protocol within the MSFD assessment across the participating parties.

The structure imposed by the IQ-ES framework requires that the quality assessor maintains focus on what each IQ represents, and the provision of information about each assessment in a transparent manner, easily understandable by a third party. These characteristics are seen as being particularly useful in the implementation of the MSFD, in which at least some cross-border use of the same indicators will no doubt be necessary to ensure consistency within a standardized assessment. For instance, this quality assessment protocol (and particularly the tabular reporting of the IQ-ES assessment) is well placed to support the call of the Intersessional Correspondence Group on the Coordination of Biodiversity Assessment and Monitoring of the OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic, to ensure consistency in the testing of all common indicators. Indeed, the format for testing of candidate biodiversity indicators developed by that group fits well with the assessment structure presented here. In this study, as a starting point, we have applied this testing protocol successfully for three distinct descriptors (1, 5, and 6). Further testing could support its applicability to the other eight descriptors.

Scoring allows for similar indicators to be separated based on an objective analysis of their overall performance with regard to the aims of the MSFD assessment. This would allow MSFD parties considering candidate metrics available to them within their assessment region to determine their readiness to assess each descriptor of GEnS. To ensure continuity of the assessment between involved parties, the scoring system used for the quality assessment should exclude as much as possible user subjectivity, and the binary system used here could be seen as its simplest form. We compared this system with the three-way scoring system (ICES, 2013a,b) within the worked examples. For instance, the two benthic vegetation candidate indicators compared exhibited similar spread using both scoring systems (worked example I). It therefore seems that, despite the relatively higher complexity and subjectivity of the three-way scoring system compared to the binary system, the ability to discriminate quality between candidate metrics did not increase. Further testing could be used to determine the relative merit of the two systems within a wider basis of ecosystem components, descriptors and pressures considered by the MSFD, but our overall assessment is that the binary system would be preferred if the aim is to reduce user subjectivity in the quality evaluation.

Although a standardized approach is seen as being necessary to objectively assess the quality of GEnS indicators in support of the MSFD, additional weight associated with IQs 1 and 3 is acknowledged here ("scientific basis" and "responsiveness to pressure," the one-out-all-out criteria). I.e., failing these IQs is seen here to preclude a failure to meet essential quality standards required for MSFD implementation. We recommend that even when IQs 1 and 3 are fulfilled, an indicator meeting only half or less of the IQs should, however, probably not be considered for implementation, unless no better alternatives exist. Overall, one of the main benefits of using quality scoring is that a minimum score could potentially be defined as the minimum quality standard below which the evaluated metric is not a suitable route to support the MSFD assessment. We suggest that this threshold could be 4 because a candidate indicator with a lower score only meets less than half of the components of quality desired within the assessment community. However, we stress that the use of this framework is not intended to define what is or is not an adequate

GEnS indicator or to determine the outcome of the selection procedure, which will be constrained by a number of additional parameters and aims. What the IQ-ES framework provides is a transparent, standardized structure to enable comparison of the quality of candidate indicators and in this way support the decision making process leading to indicator selection.

The objective quality testing protocol suggested here, and the standardized format for the reporting of this assessment we propose, could guide parties seeking better indicators for a given descriptor toward solutions in indicators scoring high in quality in other regions, and further support consistency of the assessment across parties. Through its structure, the use of the IQ-ES framework could help to inform about what types of additional information or method development are lacking within the assessment of individual parties, once local-specific constrains have been identified.

We identify IQs 3 and 4 ("responsiveness to pressure" and "possibility to set targets") as potential stumbling blocks in the quality assessment, and thus the comparison and selection of indicators. The outcomes of the evaluations of these two criteria may be more dependent upon the choice and adequacy of the analytical approaches employed, than on the indicator and data used in those assessments. Issues such as comparability of datasets between systems, the identification of effect sizes that account for natural variability, non-linear pressure-response relationships, uncertainty and spatial and temporal autocorrelation may require the use of robust quantitative data analysis methods. Generalized additive modeling (Hastie and Tibshirani, 1990), generalized linear models (Dobson, 2001), mixed effects modeling (Pinheiro and Bates, 2000), Meta-analysis statistics (Borenstein et al., 2011), mechanistic modeling and data assimilation (Hyder et al., 2015) and many other methods are therefore likely to be needed in many instances. In addition, high frequency data (e.g., those based on remote sensing) may require the application of suitable techniques such as spectral methods, to identify harmonic structures (Bloomfield, 2004). Whether the analysis technique used is adequate to the complexity of data at hand, the IQ tested for, the scale covered by the analysis (e.g., local c.f. regional), and the resources and expertise available in each case are therefore seen as essential components of the quality assessment of indicators with regard to these two criteria.

Finally, despite its timeliness and contribution toward objectivity within the MSFD indicator selection process, this study is not sufficiently comprehensive to cover the diversity of data, indicator, pressure, and habitat types associated with the 11 GEnS descriptors. However, it highlights important aspects requiring consideration within the assessment, which will only be as good as the indicators chosen and the strategies employed to monitor GEnS. Overall, standardized approaches such as this will be required to ensure consistency, and facilitate cross-border development and the sharing of knowledge during the MSFD implementation.

AUTHOR CONTRIBUTIONS

AQ, DK, JS, KM, PS, JB, and JC conceptualized the manuscript. DK, AB, JC, RC, HN, SC, and AQ provided the worked examples. All authors contributed to the text.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmars.2016.00073

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Light Thresholds to Prevent Dredging Impacts on the Great Barrier Reef Seagrass, *Zostera muelleri* ssp. *capricorni*

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Coastal seagrass habitats are at risk from a range of anthropogenic activities that modify the natural light environment, including dredging activities associated with coastal and port developments. On Australia's east coast, the tropical seagrass *Zostera muelleri* ssp. *capricorni* dominates intertidal mudbanks in sheltered embayments which are also preferred locations for harbors and port facilities. Dredging to establish and maintain shipping channels in these areas can degrade water quality and diminish light conditions that are required for seagrass growth. Based on this potential conflict, we simulated *in-situ* light attenuation events to measure effects on *Z. muelleri* ssp. *capricorni* condition. Semi-annual *in situ* shading studies conducted over 3 years were used to quantify the impact of prolonged light reduction on seagrass morphometrics (biomass, percent cover, and shoot density). Experimental manipulations were complimented with an assessment of 46 months of light history and concurrent natural seagrass change at the study site in Gladstone Harbour. There was a clear light-dependent effect on seagrass morphometrics during seagrass growing seasons, but no effect during senescent periods. Significant seagrass declines occurred between 4 and 8 weeks after shading during the growing seasons with light maintained in the range of 4–5 mol photons m^{-2} d^{-1}. Sensitivity to shading declined when applied in 2-week intervals (fortnightly) rather than continuous over the same period. Field observations were correlated to manipulative experiments to derive an applied threshold of 6 mol photons m^{-2} d^{-1} which formed the basis of a reactive light-based management strategy which has been successfully implemented to ensure positive ecological outcomes for seagrass during a large-scale dredging program.

Keywords: seagrass, shading, light attenuation, thresholds, dredging management, *Zostera muelleri*, indicators

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INTRODUCTION

Seagrasses cover 38,079 km^2 of habitat on Australia's east coast within the boundary of the Great Barrier Reef World Heritage Area (GBRWHA; Coles et al., 2015). Coastal seagrasses are an integral part of the health and ecosystem function of the GBRWHA and provide key habitat linkages, feeding grounds for globally threatened turtles and dugong, habitat for commercially important fisheries, sediment trapping and stabilization, effective nutrient filtering from coastal inputs, and carbon sequestration (Hemminga and Duarte, 2000; Jackson et al., 2001; Orth et al., 2006; Romero et al., 2006; Heck et al., 2008; Duarte et al., 2010). Despite being highly valued globally for their contribution to ecosystem services, seagrass habitats are threatened by a range of anthropogenic activities including coastal development and declining water quality from poor catchment management activities (Waycott et al., 2009; Grech et al., 2012; Costanza et al., 2014). Anthropogenic pressures on seagrasses are often compounded by natural events such as severe storms and flooding that may cumulatively lead to widespread seagrass decline. This has occurred on the tropical and sub-tropical east coast of Australia where severe tropical storms have contributed to widespread seagrass declines in recent years (Devlin et al., 2012; Rasheed et al., 2014).

A major cause of seagrass losses globally relates to human induced changes to the inshore environment that reduce available light, the primary driver of seagrass growth and distribution (Dennison, 1987; Duarte, 1991; Ralph et al., 2007). The risk of these types of impacts along the Great Barrier Reef (GBR) coast tends to be highest in areas where urban development and port infrastructure have a strong foothold (Grech et al., 2011). In the GBRWHA, extensive seagrass meadows commonly occur in proximity to large port facilities (Grech and Coles, 2010). Recent, well-publicized port expansions (BREE, 2012; Grech et al., 2013) place adjacent seagrass meadows under increased pressure. The capital works required for port developments can include large-scale dredging programs, which can have negative impacts on seagrass through direct burial and/or physical removal, and indirectly from turbidity plumes and the associated reduction in available light (Erftemeijer and Robin Lewis, 2006). In the GBRWHA, recent studies have shown that these plumes can have a substantial impact on seagrass (York et al., 2015). While physical damage to seagrass is relatively easy to quantify or directly avoid, it is the potential for large and persistent sediment plumes which are much harder to effectively forecast the scale of impact or to mitigate against seagrass loss.

The impact of dredge plumes are typically managed using measures not directly related to the ecological requirements of marine plants, such as reference to a background level of turbidity (Sofonia and Unsworth, 2010). Using the plant's light requirements to ensure minimal impacts is seldom attempted, largely due to a lack of understanding on what the *in situ* light requirements are for most seagrass species (Ralph et al., 2007). Turbidity can provide a measure of added pressure from dredging activity to the ecosystem, but does not necessarily have any direct biological relevance or account for the in-built resilience of an organism or whole system over short timescales (Sofonia and Unsworth, 2010). Adopting a direct measure of available light as a threshold for seagrass management is directly related to the plant's growth requirements making it far more preferable to turbidity.

Determining an appropriate light threshold for seagrasses involves several challenges: the light environment can be naturally highly variable over multiple timescales; plants can have dramatically different light requirements depending on time of year (Staehr and Borum, 2011); seagrasses can tolerate periods of time below their minimum light requirement without long-term impacts; and a range of other environmental parameters including water temperature and sediment chemistry can further influence *in situ* light requirements (Koch, 2001; Lee et al., 2007). The plant response to fluctuating light begins with explicit gene regulation driving changes in photosystems and pigment composition before growth rates and eventual plant morphology or meadow scale reductions become apparent (Abal et al., 1994; Collier C. J. et al., 2012). While laboratory experiments have helped to resolve the fundamental timeline of many of these responses (Abal et al., 1994; Collier C. J. et al., 2012; McMahon et al., 2013), the actual timeline of *in situ* seagrass growth dynamics is likely to be quite different due to additional extrinsic factors that cannot easily be replicated in laboratory or mesocosm trials such as nutrient availability, water temperature, hydrodynamics, epiphyte loads, water column oxygen fluxes and sediment chemistry (Carruthers et al., 2002; Waycott et al., 2005; Raun and Borum, 2013). *In situ* shading studies provide an empirical approach to measuring impacts of prolonged incident light attenuation and identify potential warning signs of decline in meadow-scale seagrass health as related to dredging or other anthropogenic-induced light reduction under realistic field conditions (Longstaff and Dennison, 1999; Collier C. et al., 2012).

Identifying the relevant timeframe to elicit a negative response by local seagrasses is a key component of developing a regionally-specific light threshold. Most seagrasses can tolerate periods of time below their minimum light requirement without long-term impacts (Alcoverro et al., 1999; Collier C. J. et al., 2012). Short-term re-allocation of carbon from storage tissues and adjustments to photosynthetic machinery can help bide time until conditions improve (Alcoverro et al., 2001; Cayabyab and Enríquez, 2007). A light threshold must establish the juncture at which compensatory physiological mechanisms are superseded by plant-scale declines (Collier C. J. et al., 2012). An applied light management strategy must consider the light quantity, quality and duration of light that is required to sustain local seagrass populations.

Many coastal seagrass species are well-adapted to the variable conditions that occur in a near-shore environment, including naturally turbid waters related to runoff, large tidal fluxes, complex hydrodynamics and oscillating temperatures creating constantly shifting optical and metabolic challenges (de los Santos et al., 2010; Collier et al., 2011; Petrou et al., 2013). Strategies to tolerate temporary light reduction are broadly the same for all species: adjusting light harvesting capacity and the efficiency of light use (Abal et al., 1994; Enriquez, 2005); adjustments to rates of growth and plant turnover (Collier et al., 2009; Collier C. J. et al., 2012; and drawing upon carbohydrate reserves to maintain a positive carbon balance (Burke et al., 1996; Touchette and Burkholder, 2000). While seagrasses adapted to marginal environments may be tolerant of wide fluctuations in light, they can also be acutely sensitive to reductions in light beyond the natural range of conditions (Ralph et al., 2007). When light drops below a critical level, seagrass productivity is compromised and significant physiological, biochemical and structural changes begin to take place eventually manifesting into broader meadow-scale losses with consequences for ecosystem function (Lee and Dunton, 1997; Ralph et al., 2007; Hughes et al., 2008).

Zostera muelleri ssp. *capricorni* is a key coastal seagrass species found along the tropical east coast of Australia (Waycott et al., 2004) and occurs in the muddy, inshore estuarine environments few other seagrass species inhabit (Lee Long et al., 1993; Carruthers et al., 2002). In port areas of the GBRWHA it is often the dominant species present, including in the Gladstone region, where it is found in monospecific intertidal meadows covering up to 40 km^2 within the port limits (Thomas et al., 2010; Supplementary Figure 1). With no known functional replacement, a large-scale dieback due to a stress event such as dredging could have wider implications for the ecological success of the inshore marine community.

The goal of this study was to develop a species-specific, light threshold for the effective management of *Zostera muelleri* ssp. *capricorni* in Gladstone, Australia. Recent expansion of port infrastructure and shipping channels around Gladstone has involved large-scale dredging and the removal of ~26 million m^3 of sediment over 3 years. *In situ* shading studies were used to elicit a response in a local seagrass population to determine a light threshold at which seagrasses will decline and over what time scale a decline is detectable in plant abundance. The approach used does not attempt to simulate a given dredging scenario but rather to apply information on how locally-adapted seagrasses withstand constant light attenuation or how regular short-term reprieves from light attenuation events affect the overall seagrass condition and its' recovery in order to better manage threats from dredging related turbidity plumes. This information was used to apply a management-based light threshold to protect seagrasses from light stress during dredging. Long-term monitoring of the seagrass meadow at an adjacent site also provided information on the status and trend of local seagrass in relation to seasonality, light history, and water temperature. The adjacent site also provides a testing ground to assess the suitability of our light threshold against seagrass condition over the long term.

Our study focused on the development of locally-relevant light thresholds that can be applied for effective management of coastal and port development activities in a way that maintains seagrass health. The term threshold, as used here, is defined as the point at which a change in external conditions causes a significant negative change in seagrass physical condition, i.e., above-ground biomass, cover, or shoot density. It is important to note that this is different to defining a minimum light requirement (MLR) for effective seagrass photosynthesis. Rather, the goal is focused around developing a biologically relevant management tool, which incorporates other local environmental drivers such as tidal cycles, seasonality and sediment chemistry dynamics that influence seagrass condition together with light *in vivo*.

MATERIALS AND METHODS

Shading Study Experimental Design

This study was conducted at Pelican Banks, Gladstone Harbour (151° 18′ 30″E, 23° 45′ 58″S), Australia (see Supplementary Figure 1) from 2010 to 2013. At Pelican Banks the tropical subspecies *Z. muelleri* ssp. *capricorni* forms a predominantly monospecific intertidal seagrass meadow on intertidal mud banks. Studies were carried out during two growing seasons for local seagrasses (ca. July to December) and two senescent seasons (ca. January to June) when seagrasses naturally decline with the onset of the tropical monsoon and subsequent cooler months in the austral winter (Mellors et al., 1993; McKenzie, 1994). Studies are described accordingly: growing seasons 1 and 2 (G1 and G2) and senescent seasons 1 and 2 (S1 and S2). The study location was chosen for its accessibility, semi-firm sediment composition for repeated measurements during emergence at low tide without compromising site integrity, and year-round seagrass cover to assess seasonal effects. A semi-diurnal tide cycle with a maximum range of 5 m meant seagrasses were exposed at least fortnightly, depending on the time of year.

The study site was ~30 × 20 m with experimental plots randomly assigned to each of three shade treatments or as controls ($n = 4$). Vertical isolation borders (sever root connection between shaded and non-shaded areas) were inserted for the shade experiments by hammering 0.25 m^2 quadrats with a 0.25 m depth into the sediment until flush with the sediment surface to isolate plots where seagrass would be measured. This ensured seagrass outside of the experimental plot could not translocate nutrients/carbohydrates to seagrass within treatment plots. Plots were also "gardened" around the isolation border perimeter prior to each sampling event to prevent seagrass growing over the border and into experimental plots. Aluminium frames were secured into the sediment and covered with 1 m^2 neutral density polyethelene shade cloth of varying intensities fixed 0.15 m above the sediment surface. Shade treatments were used to assess three levels of reduced light on seagrass health; high, medium and low shade, equivalent to ~15, 30, and 45% of incident benthic light, respectively. Control plots were established using quadrats with steel frames and isolation borders but without shade screens. No control was used for the effect of rhizome severing based on the work of Rasheed (1999) which found no border effect using an identical experimental design and field materials to measure shading effects on the same species. Controlling for the additional effect of shade screens on water movement was not possible without creating additional shading or fouling over control plots (see Fitzpatrick and Kirkman, 1995). Shade screens were changed and cleaned fortnightly to reduce the effects of fouling on shade treatments. Light intensities under shade treatments fluctuated with natural insolation but maintained consistent patterns among treatments and relative differences to naturally occurring benthic light, indicating that fouling of the shade screens was minimal. Shade screens were removed at the end of each experiment to track potential recovery from treatment conditions.

Experimental plots were randomly assigned to varying durations of continuous shading (between 1 and 3 months) during each seasonal study (**Table 1**). This variation in shading study duration and tracking of recovery was necessary to align the program with expected timeframes for managing impacts to seagrass health during dredging operations as required by managers and regulators. Therefore, comparison among seasonal

TABLE 1 | Shading study design during senescent seasons 1 and 2 (S1, S2) and growing seasons 1 and 2 (G1, G2).

Study	Date Commenced	Shading Duration	Shade Treatments	*N*
S1	May 2010	1 month	H, M, L, C	4
G2	Sept 2010	3 months and fortnightly	H, M, L, C	4
S2	May 2012	3 months	H, M, L, C	4
G3	Sept 2013	3 months	H, M, L, C	4

Shade treatments included high shade (H), medium shade (M), low shade (L), and control (C). N is the number of replicates per shade treatment for each study.

studies was limited to shading durations comparable between studies. In addition, fortnightly cyclic shading was carried out during G1 to assess the impact of periodic turbidity plumes (i.e., shorter periods of reduced light and subsequent respites) on seagrass condition.

Light Climate

Light (photosynthetically active radiation, PAR) was measured within the seagrass canopy and under shade treatments using 2π cosine-corrected irradiance loggers (Submersible Odyssey Photosynthetic Irradiance Recording System, Dataflow Systems Pty. Ltd., New Zealand) calibrated using a cosine corrected Li-Cor underwater quantum sensor (LI-190SA; Li-Cor Inc., Lincoln, Nebraska USA) and corrected for immersion using a factor of 1.33 (Kirk, 1994). Loggers were deployed on site for the duration of shading and maintained using automated wiper units. Readings were made at 15 min intervals and used to measure total daily light (mol photons m^{-2} day^{-1}) reaching seagrasses under each shading treatment.

Substantial tidal flux in Gladstone Harbour leads to dramatic shifts in daily light intensities on the intertidal banks due to fortnightly intertidal exposure cycles and this has the potential to control light availability to the plant (Koch and Beer, 1996). To evaluate light over a practical timeframe for measuring impacts, light data was integrated as a rolling 14 day mean of the total daily benthic light under each shading treatment, controls, as well as the long-term monitoring site (detailed below). Current understanding of seagrass response indicates under low light stress conditions, physiological adjustments first occur over a matter of days, whereas plant-scale changes take place after a number of weeks and are a reflection of the integrated light history over that period rather than short term daily fluxes (McMahon et al., 2013). This 2 week rolling average incorporated spring and neap tide conditions, variation in tide height, and the associated degree of exposure that affects the light conditions reaching the seagrass. An assessment of integrated light over a 2-week period is therefore in line with both tidally-driven fluxes in light, as well as a period of time preceding apparent morphological changes to seagrass.

Seagrass Morphometrics

Seagrass above-ground biomass, percent cover and shoot density were measured at fortnightly or monthly intervals in each treatment plot during S1 and G1 studies, while only biomass and percent cover were recorded during S2 and G2 studies. Above-ground biomass was measured using a "visual estimates of biomass" technique (Kirkman, 1978; Mellors, 1991; Rasheed, 1999). Biomass was estimated for each plot by an experienced observer recording a rank of seagrass biomass from photographs of each plot taken during sampling. Biomass ranks were assigned in reference to a series of photographs of similar seagrass habitats for which above-ground biomass has previously been measured. The same observer was used for the duration of each study to remove any inter-observer variability. At the completion of recording ranks, the observer ranked a series of additional photographs that had been previously harvested, dried, and weighed and which represented the range of seagrass biomass in the survey. A regression of ranks and biomass from these calibration quadrats was generated for each observer ($r^2 = 0.97$; see Supplementary Figure 2) and applied to the measuring plot ranks to determine above-ground biomass estimates. Biomass ranks were then converted into above-ground biomass estimates in grams dry weight per square meter (g DW m^{-2}). Shoot density was estimated by counting all shoots within a mini-quadrat (0.01 m^2) randomly placed three times in each measuring plot except where total-plot shoot density was less than 30 shoots and all shoots were counted within the 0.25 m^2 plot. Seagrass percent cover estimates were made for each plot by an observer using a standardized photo guide sheet.

Light History, Environmental Conditions and Seagrass Trend in the Meadow

A monitoring site was established in the *Z. muelleri* ssp. *capricorni* meadow adjacent to the shading study site to assess incident light and temperature at the seagrass canopy and its potential influence on seagrass meadow condition over longer time scales under natural harbor conditions. Light was recorded continuously between November 2009 and September 2013. Light loggers were deployed and operated in the same manner as in the shading studies through June 2012. From July 2012, irradiance loggers were replaced with LiCor underwater sensors with inbuilt wiper units and customized telemeted systems (Vision Environment QLD., 2013) to ensure continuous data collection and immediate availability of data during dredging operations. Water temperature was measured in the seagrass canopy (Thermodata Pty Ltd, Melbourne, Australia), daily rainfall (Bureau of Meteorology Australia[1]) and total hours of daytime tidal air exposure of the meadow (Maritime Safety Queensland, Department of Transport and Main Roads) were also collected.

Seagrass condition was assessed at three 50 m transects nested in two 50 x 50 m sites. Sites were selected within a relatively homogenous section of the *Z. muelleri* ssp. *capricorni* meadow. Seagrass above-ground biomass was estimated within a 0.25 m^2 sampling quadrat placed at 0 m and then every 5 m along each transect (eleven sampling points per transect) using the same technique described above (observer regression of ranks, $r^2 = 0.95$). Mean biomass was calculated for each sampling event ($n =$

[1]www.bom.gov.au

66 quadrats) with change in biomass calculated from consecutive sampling events.

Data Analysis

All values displayed are means ± standard error (SE). Differences in morphological responses of seagrass among shading treatments and over time were assessed using repeated measures analysis of variance (rmANOVA). Data were checked for homogeneity of variance by assessing residual plots. Significant deviations from normal variance were found in G1 biomass data which were log-transformed prior to analysis. If data still did not meet the criteria, the *p*-value was set to 0.01 to minimize the risk of a Type I error (Underwood, 1997). For repeated measures ANOVAs, matrices were tested for sphericity using Mauchly's test. If the assumption of sphericity was not met ($p < 0.05$) the Greenhouse-Geisser (G-G) epsilon adjustment was applied to the numerator and denominator degrees of freedom. Differences among treatment effects at a given sampling time were compared using Tukey's *post-hoc* analysis. For data collected during the "recovery phase," a one-way ANOVA was performed when a single recovery time point was measured with shading intensity as a fixed effect and tests for homogeneity of variance and transformation applied as previously described. Statistical analyses were performed using Statistica 7.0. When multiple recovery period measurements were taken, rmANOVA methods as described for the shading period were applied.

RESULTS

Seagrass Morphometrics

Shading treatments did not have a significant effect on *Z. muelleri* ssp. *capricorni* morphology during either senescent season study (S1 and S2). However, after 1 month of shading there was a significant increase in shoot density during S1 ($p < 0.05$), but no significant changes in biomass or percent cover ($p > 0.05$, **Table 2**; **Figures 1–3**). Above-ground biomass and percent cover declined significantly over the 12 weeks of shading among all treatments during S2 (both $p < 0.001$); significantly lower above-ground biomass and percent cover in treatments compared to

TABLE 2 | Repeated measures ANOVA of the effects of shading treatment (among groups effect) and time (within groups effect) for biomass, percent cover and shoot density during senescent seasons 1 and 2 (S1, S2) and growing seasons 1 and 2 (G1, G2).

	df	*F*	*p*		*df*	*F*	*p*
	S1				**G1**		
Above-ground biomass				**Above-ground biomass^**			
Shade	3	0.49	ns	Shade	3	13.31	***
Time	1	1.70	ns	Time	4	137.62	***
Shade × Time	3	0.001	ns	Shade × Time	12	12.87	***
Percent cover				**Percent cover**			
Shade	3	1.03	ns	Shade	3	3.21	ns
Time	1	2.32	ns	Time	4	72.56	***
Shade × Time	3	1.54	ns	Shade × Time	12	6.13	***
Shoot density				**Shoot density**			
Shade	3	0.30	ns	Shade	3	0.49	ns
Time	1	7.58	*	Time	4	21.55	***
Shade × Time	3	0.17	ns	Shade × Time	12	2.58	*
	S2				**G2**		
Above-ground biomass				**Above-ground biomass**			
Shade	3	4.33	*	Shade	3	2.73	ns
Time	4	22.14	***	Time	5	15.05	***
Shade × Time	12	1.29	ns	Shade × Time	15	4.16	***
Percent cover				**Percent cover**			
Shade	3	4.97	*	Shade	3	4.06	*
Time	4	19.41	***	Time	5	46.79	***
Shade × Time	12	1.28	ns	Shade × Time	15	3.27	***
Shoot density†				**Shoot density†**			
Shade	–	–	–	Shade	–	–	–
Time	–	–	–	Time	–	–	–
Shade × Time	–	–	–	Shade × Time	–	–	–

*The ANOVAs were not significant (ns), or significant at *p < 0.05, **p < 0.01, ***p < 0.001. Probability values are Greenhouse-Geiser adjusted p values.*

^Log transformed;

†Not recorded.

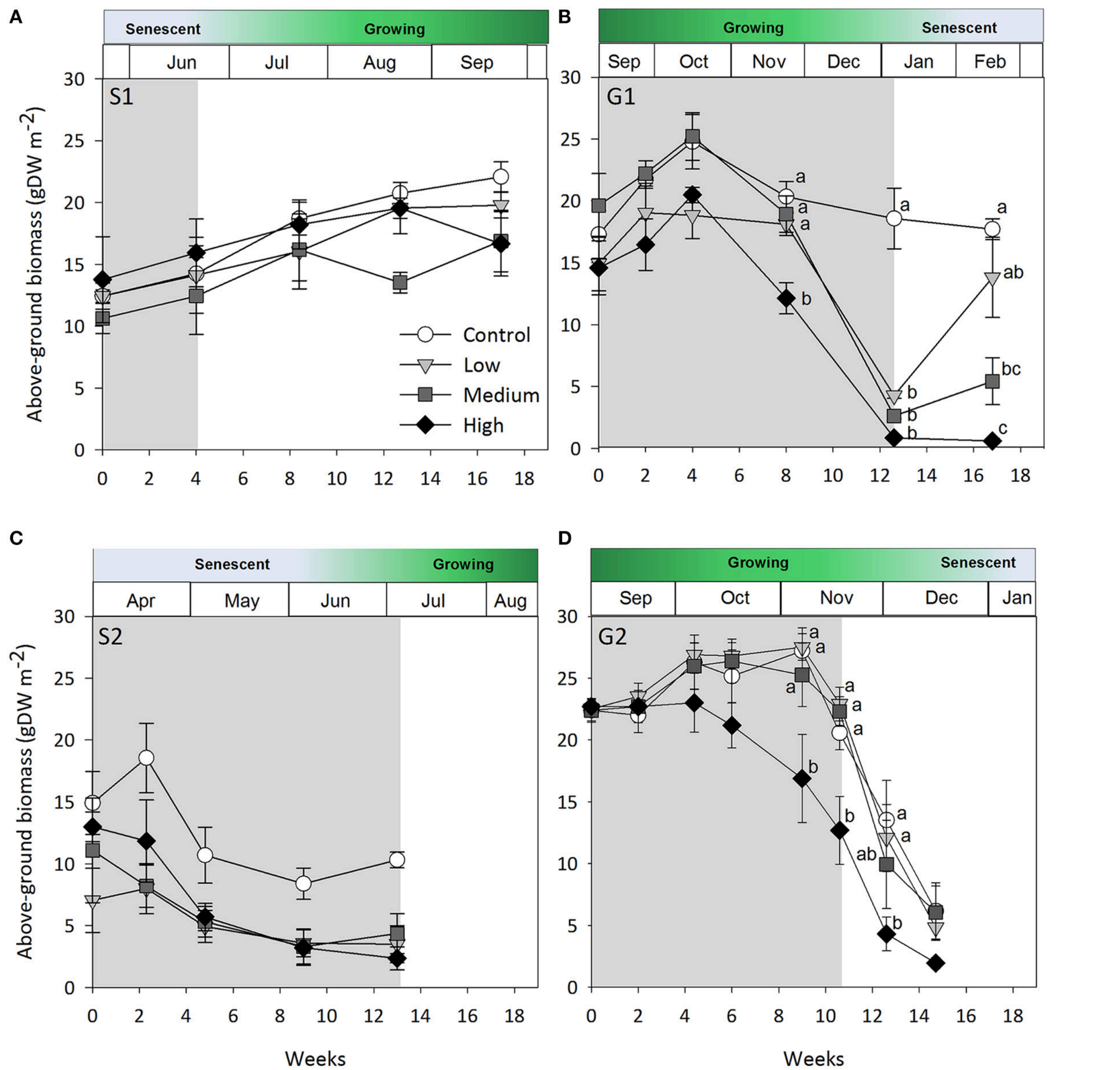

FIGURE 1 | Seagrass above-ground biomass over time. (A) Senescent season 1 (S1); **(B)** growing season 1 (G1); **(C)** senescent season 2 (S2); **(D)** growing season 2 (G2). Grayed area represents shading periods and white area represents monitored recovery periods where data was recorded. Data represent mean ± SEM ($n = 4$). Superscripted identical letters indicate no significant difference among shading treatment (control, low, medium, high) at $p < 0.05$ (Tukey's *post-hoc* test).

control plots; this was apparent from the start of the study (both $p < 0.05$, **Table 2**; **Figures 1–2**).

Shading had a detrimental effect on *Z. muelleri* ssp. *capricorni* above-ground biomass during the growing seasons (G1 and G2, shade × time interaction $p < 0.001$, **Table 2; Figure 1**). During both growing season studies, biomass was significantly lower by the 8 week sampling under high shade treatments compared to controls and other treatments (**Figure 1**). This occurred between 4 and 8 weeks in G1 and 6 and 8 weeks in G2. There was significant loss of above-ground biomass under all treatments compared to control plots by 12 weeks during G1, including near total loss of above-ground biomass under high shade plots (**Figure 1B**). Within 4 weeks of shade removal, above-ground biomass under low shade treatments recovered to control levels, whereas biomass under medium and high shade treatments remained significantly lower than control plots ($p < 0.001$; **Figure 1B**). Control plots did decline somewhat from a peak at 4–16 week measurements, likely due to the onset of characteristic seasonal senescence which occurred toward the end of the study (Jan–Feb 2011). Similarly, above-ground biomass under high shade was significantly lower than under control, low and medium shade treatments by 8 weeks of shading during G2. Declines in above-ground biomass and percent cover from mid-November in G1 and G2 across controls and all treatment

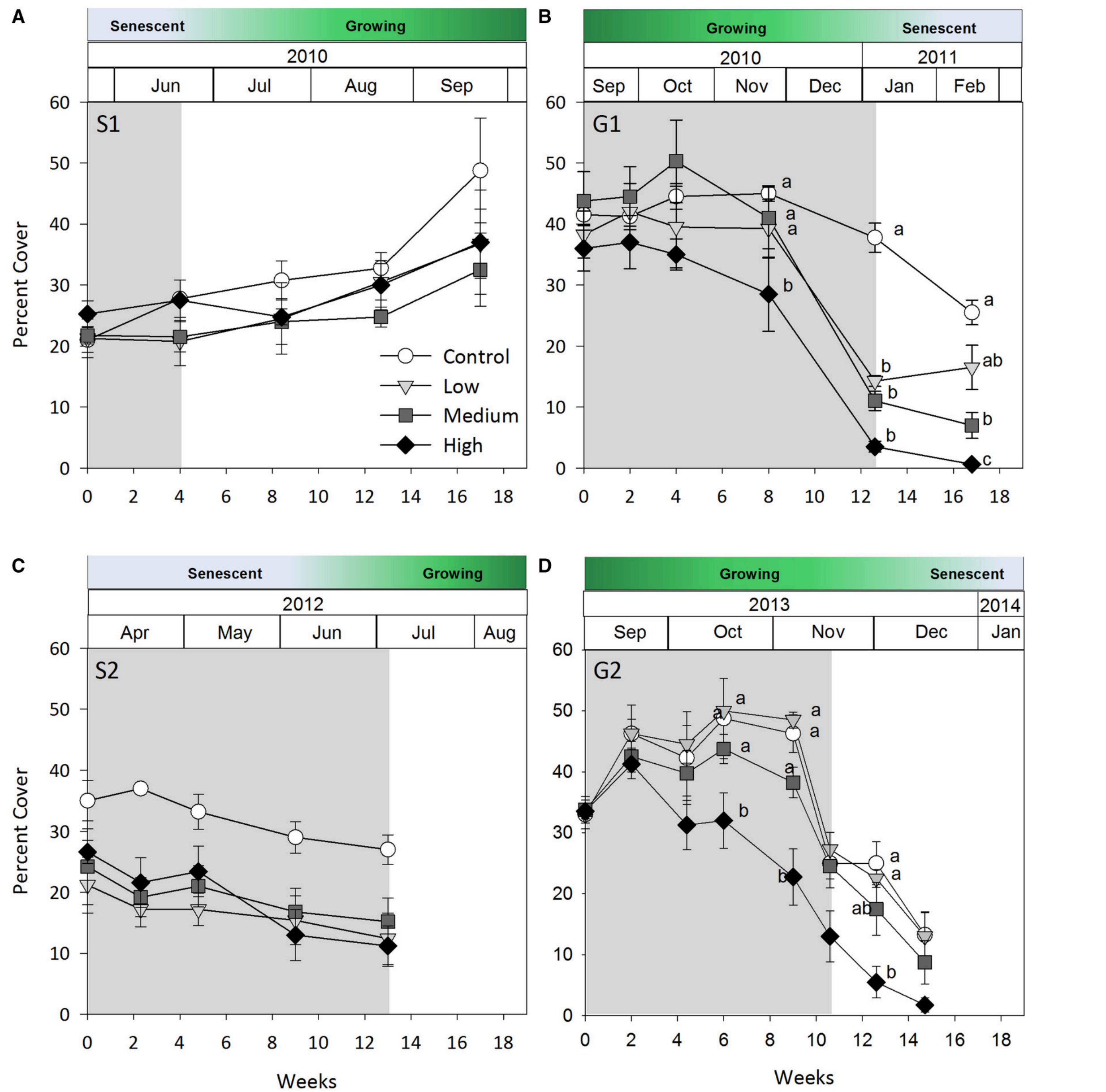

FIGURE 2 | Seagrass percent cover over time. (A) Senescent season 1 (S1); **(B)** growing season 1 (G1); **(C)** senescent season 2 (S2); **(D)** growing season 2 (G2). Grayed area represents shading periods and white area represents monitored recovery periods where data was recorded. Data represent mean ± SEM ($n = 4$).

plots are consistent with seasonal declines with the onset of the senescent season (**Figures 1B,D**, **2B,D**).

Negative effects of shading on percent cover during both growing seasons were similar to those recorded for above-ground biomass (both p-values for shade × time interaction <0.001, **Table 2**; **Figure 2**). Percent cover was significantly lower under high shade treatments compared with control, low and medium shade treatments for G1and G2 within 8 and 6 weeks, respectively, (**Figures 2B,D**). Within 12 weeks percent cover under all shade treatments was significantly lower than control plots during G1 (**Figure 2B**). Recovery of seagrass during G1to a percent cover similar to control plots occurred within 4 weeks of shades being removed for the low shade treatment, but there were no similar signs of recovery for treatments that had been under medium or high shade treatment (**Figure 2B**; **Table 3**). Percent cover of seagrass under high shade similarly demonstrated no sign of recovery 2 weeks following shade removal during G2 (**Figure 2D**; **Table 3**). High shade plots were nearly devoid of seagrass cover 4 weeks after shade removal for G1 and G2 (**Figures 2B,D**).

Shoot density was less sensitive to shading than percent cover and above-ground biomass. Seagrass shoot density decreased significantly by 12 weeks under the high shade treatment compared with control and low shade treatment plots during the

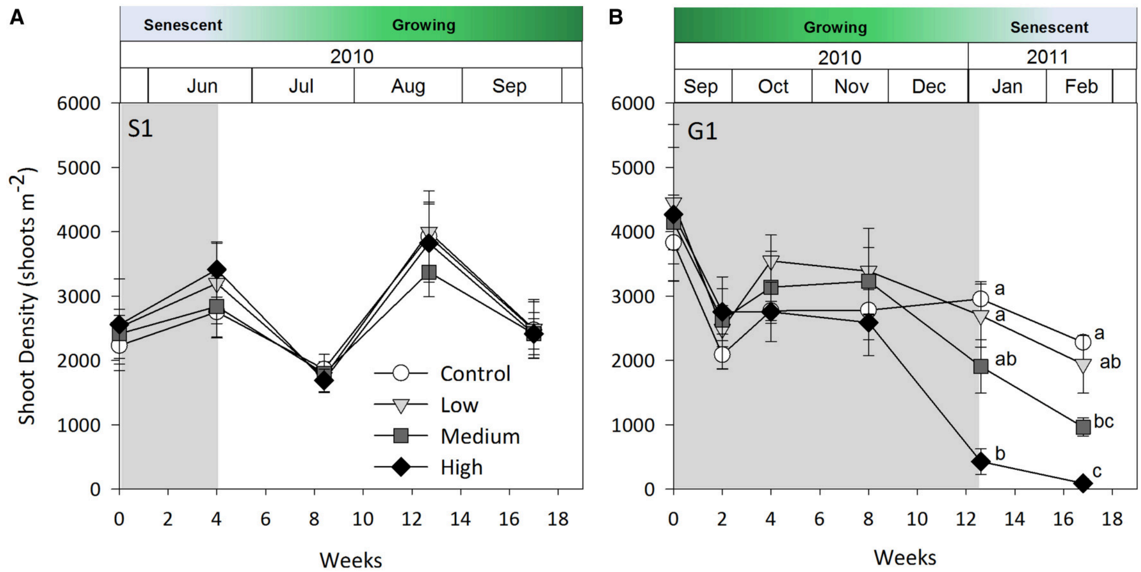

FIGURE 3 | Seagrass shoot density over time. (A) Senescent season 1 (S1); **(B)** growing season 1 (G1); note shoot density not recorded during S2 or G2 (see Results). Grayed area represents shading periods and white area represents monitored recovery periods where data was recorded. Data represent mean ± SEM ($n = 4$).

growing season (G1 study, shading x time interaction $p < 0.05$, **Table 2**; **Figure 3B**). There were no signs of recovery to control levels 4 weeks after shades were removed (**Figure 3B**). Shading had no significant effect on temporal fluctuations in shoot density during the senescent season (S1 study, $p > 0.05$, **Table 2; Figure 3A**).

Seagrass was less sensitive to fortnightly cyclic shading than to continuous shading when tested during G1. Above-ground biomass data is only presented, but shoot density and percent cover results were analogous. Above-ground biomass under all shade treatments was similar to control plots for the first 8 weeks of the study; however, by week 12 biomass under all shade treatments was equally and significantly lower than under control plots (two-way rmANOVA, shade x time interaction, $p < 0.01$, **Figure 4**). After 4 additional weeks without shading (weeks 12–16), no biomass recovery occurred under high shade treatments relative to controls ($p < 0.05$). While seagrass loss was delayed under cyclic shading, the magnitude of impact of these treatments was similar to those found under continuous shading after 12 weeks.

Above-ground biomass and percent cover in control plots throughout all studies was similar to that measured at the nearby long-term monitoring site (see **Figure 6**) indicating no effect of the physical presence of frames holding shade screens otherwise on the experiment.

Light Climate in Relation to Morphometric Results

During both senescent season studies (S1 and S2), light levels were strongly attenuated under all shade treatments compared to controls, while no measured loss of seagrass biomass, percent cover or shoot density was recorded after 4 and 13 weeks, respectively, when shades were in place (**Figures 5A,C**). Light intensities measured under S1 and S2 shades were generally between 2 and 6 mol photons m^{-2} d^{-1}, a similar range recorded during the G1 study under the same shading treatments.

During the first growing season (G1), light intensities under the high shade treatment measured consistently below 2 mol photons m^{-2} d^{-1} leading to significant declines in above-ground biomass and percent cover recorded by 8 weeks (**Figure 5B**). Light remained at or below 2 mol photons m^{-2} d^{-1} for the remaining 4 weeks of shading over which time seagrass was completely lost from high shaded plots. Light under medium shade treatments was higher and more variable over the course of G1, but generally stayed above 4 mol photons m^{-2} d^{-1} for the initial 10 weeks of the study, while light under low shades remained above 6 mol photons m^{-2} d^{-1} during the same period. Light declined between weeks 10 and 12 of the experiment across controls and all treatments during a period of high rainfall in November and December 2010 (Australian Bureau of Meteorology[2]). Light levels were consistently below 4 mol photons m^{-2} d^{-1} under all shade treatments in the fortnight leading up to the 12 week sampling event, when biomass and percent cover were significantly lower for all treatments compared with control plots (**Figure 5B**). Four subsequent weeks with shades removed (recovery; weeks 12–16) were insufficient reprieve for biomass, percent cover or shoot density to recover under medium and high shade treatments while low shade treatments recovered when returned to ambient light conditions (**Figures 1B**, **2B**, **3B**).

[2]www.bom.gov.au/climate/data/

TABLE 3 | Repeated measures and one-way ANOVA of recovery from shading treatments (among groups effect) and time (within groups effect) for biomass, percent cover and shoot density during senescent seasons 1 and 2 (S1, S2) and growing seasons 2 (G2).

	df	*F*	*p*		*df*	*F*	*p*
S1				**G1**			
Above–ground biomass				**Above–ground biomass^**			
Shade	3	1.54	ns	Shade	3	19.58	***
Time	3	4.89	**	Time#	–	–	–
Shade × Time	9	0.83	ns	Shade × Time#	–	–	–
Percent cover				**Percent cover**			
Shade	3	2.16	ns	Shade	3	21.86	***
Time	3	8.38	**	Time#	–	–	–
Shade × Time	9	0.38	ns	Shade × Time#	–	–	–
Shoot density				**Shoot density**			
Shade	3	0.13	ns	Shade	3	17.06	***
Time	3	26.74	***	Time#	–	–	–
Shade × Time	9	0.39	ns	Shade × Time#	–	–	–
S2				**G2**			
Above–ground biomass†				**Above–ground biomass**			
Shade	–	–	–	Shade	3	1.85	ns
Time	–	–	–	Time	2	24.65	***
Shade × Time	–	–	–	Shade × Time	6	0.89	***
Percent cover†				**Percent cover**			
Shade	–	–	–	Shade	3	5.30	*
Time	–	–	–	Time	2	55.96	***
Shade × Time	–	–	–	Shade × Time	6	1.43	ns
Shoot density†				**Shoot density†**			
Shade	–	–	–	Shade	–	–	–
Time	–	–	–	Time	–	–	–
Shade × Time	–	–	–	Shade × Time	–	–	–

*The ANOVAs were not significant (ns), or significant at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Probability values are Greenhouse-Geiser adjusted p values.*
^Log transformed;
† Not recorded;
 # Not tested, one-way ANOVA applied.

During the second growing season (G2), light under high shaded plots was less than 5 mol photons m^{-2} d^{-1} in the fortnight leading up to detection of a significant decline in seagrass percent cover at 6 weeks (**Figure 5D**). Light declined further to <4 mol photons m^{-2} d^{-1} for the fortnight leading up to sampling at 9 weeks, when significant declines in percent cover and above-ground biomass were detected. Light under low and medium shade treatments mostly stayed above 5 mol photons m^{-2} d^{-1} for the duration of the G2 shading study; one exception was when light dropped below 5 mol photons m^{-2} d^{-1} under medium shade for ∼1 week at week 9; although with no detectable change in seagrass biomass or percent cover recorded. In contrast, significant declines in seagrass biomass and/or percent cover were recorded following more prolonged periods of light <5 mol photons m^{-2} d^{-1} under high shade treatments at weeks 6, 9, and 10.

Climate History and Seagrass Trend

From September 2009 to September 2013, seagrass above-ground biomass at the monitoring site followed a typical oscillating seasonal pattern. *Z. muelleri* ssp. *capricorni* reached maximum biomass between October and December each year which coincided with higher water temperatures and ambient light (**Figure 6**). Light levels in the meadow were relatively high during the growing season which paralleled net positive growth. Light intensities remained above 8 mol photons m^{-2} d^{-1}; well above the levels at which significant impacts were measured under shade treatments. Annual seagrass senescence began at approximately the start of the year when temperatures consistently reached >30°C in the meadow and the onset of rain and flooding events led to reductions in light (**Figure 6**). The relationship between seagrass above-ground biomass and mean maximum daily water temperature for the month prior to sampling in the growing period likewise indicated water temperature correlated with seagrass biomass ($p < 0.01$, $r^2 = 0.55$) until water temperature exceeded 30°C and seagrass declined, despite high light intensities over the same period. Seagrass abundance typically reached a minimum by April/May after which a return to growth and increased seagrass biomass was observed around July each year.

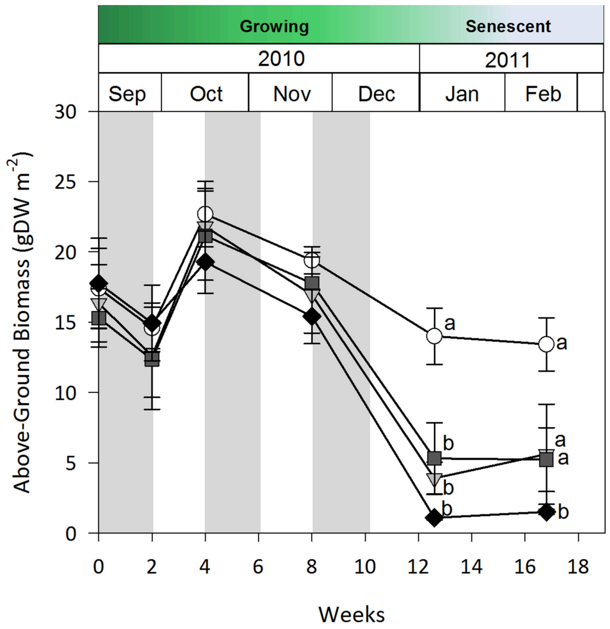

FIGURE 4 | Seagrass above-ground biomass over time during the 2-week cyclic shading experiment in growing season 1 (G1). Grayed area represents shading periods and white area represents monitored recovery periods where data was recorded. Data represent mean ± SEM (*n* = 4).

DISCUSSION

Z. muelleri ssp. *capricorni* condition (biomass, shoot density and percent cover) was measurably driven by light reductions tested during the growing seasons but was unaffected by a reduction in light applied during either senescent season. Similar field shading experiments have demonstrated time-of-year is a critical factor in defining the magnitude of the plant's response to reduced light conditions, linked to seasonal light and water temperatures (Lavery et al., 2009). We found that *Z. muelleri* ssp. *capricorni* declined in the growing season when light was $\leq$ 5 mol quanta m^{-2} d^{-1} for periods of time exceeding 4 weeks. This was successfully used to develop a conservative management threshold to protect seagrasses during dredging operations by maintaining light levels above 6 mol quanta m^{-2} d^{-1}.

The significant and consistent decline in *Z. muelleri* ssp. *capricorni* during the growing season shading studies highlights the sensitivity of this species during its period of peak productivity and expansion. *Z. muelleri* ssp. *capricorni* carbon fixation and above-ground biomass have been shown to significantly decline when grown under saturating or limiting light levels in conjunction with extreme temperatures (>33°C; Collier et al., 2011) and for temperate *Z. muelleri* when grown under 30°C conditions (York et al., 2013). Similar results have been found for the congeneric northern hemisphere species, *Zostera marina*, with summertime declines coinciding with low light and high temperatures (Zimmerman et al., 1989; Olesen and Sand-Jensen, 1993).

The high metabolic demand that comes with warmer conditions was typically supported by higher light (approximately July to December) at our study site (**Figure 6**). This likely allowed an increase in photosynthetic processes to keep up with rising seasonal temperatures up until a point, after which respiration would continue to increase without a concomitant increase in photosynthesis (Bulthuis, 1987; Lee et al., 2007). When such an imbalance occurs this can lead to die-off, whether seasonal or driven by episodic reductions in light. It was likely that *Z. muelleri* ssp. *capricorni* was not meeting its metabolic requirements during these warmer months when subjected to reduced light levels, leading to a dieback under our shading treatments. Similar trends were seen at our permanent monitoring location adjacent to the study site where seasonal cycles of seagrass growth and decline paralleled temperature and light regimes (**Figure 6**).

Seasonal seagrass growth rates are closely linked to light and temperature patterns (Lee et al., 2007). Intertidal *Z. muelleri* ssp. *capricorni* meadows along the Queensland coast follow typical seasonal fluctuations in condition linked to light, temperature and tidal exposure (Mellors et al., 1993; McKenzie, 1994; Carruthers et al., 2002; Petrou et al., 2013). From August to December, clearer waters and warmer temperatures spur rapid growth and expansion of seagrass meadows in the Gladstone region before typical dieback in late austral summer with the onset of high temperatures and wet season conditions.

The lack of a low light response in the senescent season could be due to a decrease in extrinsic energy requirements due to the lower seagrass standing crop and preferential use of carbohydrate reserves to support seagrass metabolic requirements (Burke et al., 1996; Touchette and Burkholder, 2000). Lavery et al. (2009) also found shading imposed over winter did not produce morphological changes; in contrast to their late summer results. They associated the effect of temperature on gross photosynthetic requirements of the plant to explain the disparity in seasonal effects. The saturating irradiance for photosynthesis (I_k) and respiration typically increase with temperature (Masini and Manning, 1997; Lee et al., 2007) equating to higher overall light requirements during summer growing periods compared to cooler months.

When light levels are sufficient, carbohydrate reserves are enhanced which help offset periods of high light attenuation by supporting short-term energy demands of the plant. In the first growing season study, medium shaded plots were not measurably affected until the 12 week sampling event and did not recover from losses within 4 weeks. While light under medium shaded plots during the first 10 weeks (4–5 mol photons m^{-2} d^{-1}) sustained *Z. muelleri* ssp. *capricorni in vivo*, it was likely near its' light requirement limit and may have exhausted energy reserves, making recovery unachievable in the short-term once shades were removed. Alternatively, light during G1 under low shaded plots, which received by and large > 6 mol photons m^{-2} d^{-1} during the study, likely enabled excess energy to be stored in the plant and used to support recovery when shades were removed. These differences in treatment response illustrate that conditions leading up to an acute stress event are important in determining recovery success. Ensuring light is maintained at a level that not

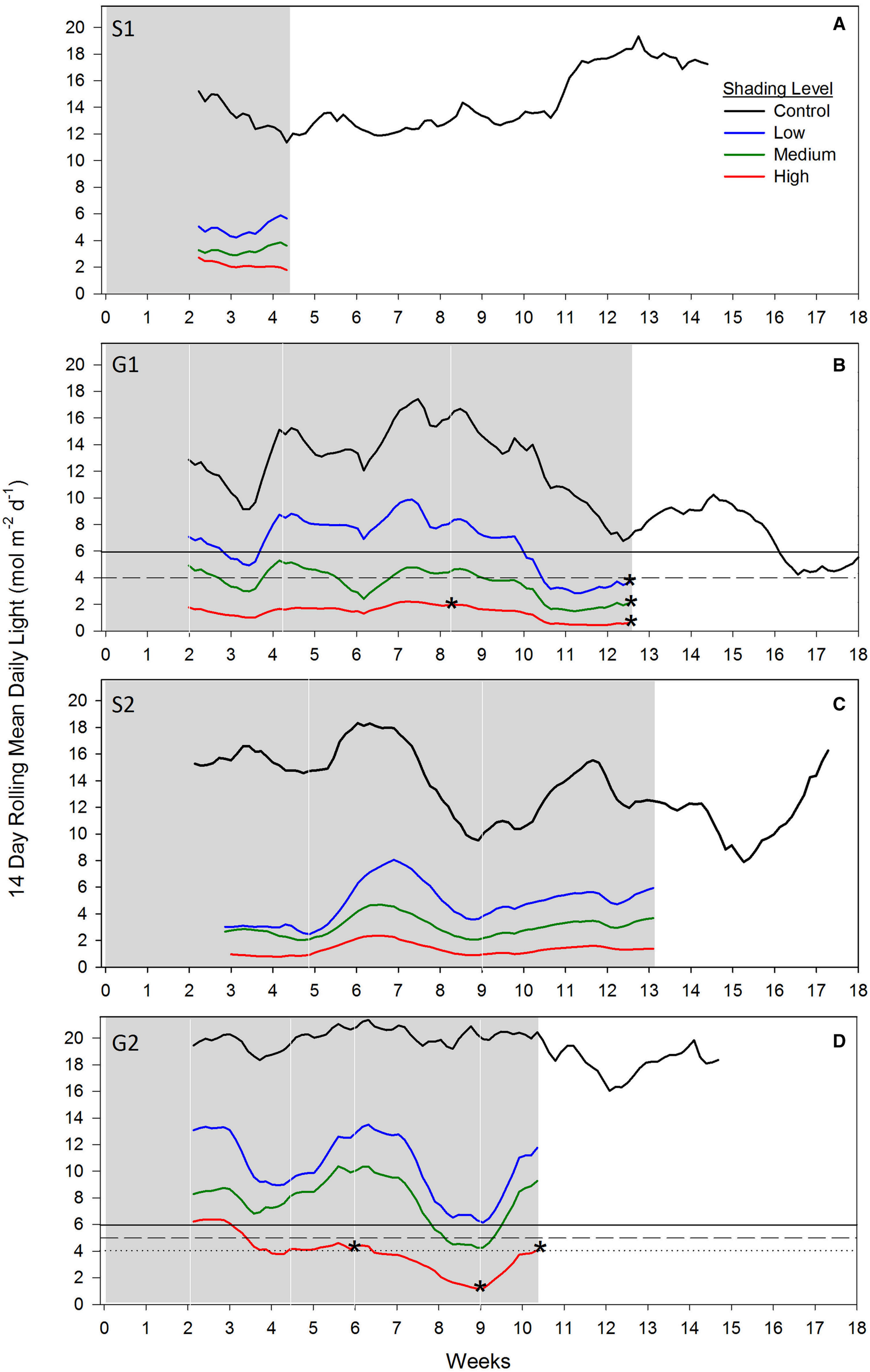

FIGURE 5 | Fourteen day rolling mean benthic light recorded under shade treatments across four shading studies. (A) Senescent season 1 (S1); **(B)** growing season 1 (G1); **(C)** senescent season 2 (S2); **(D)** growing season 2 (G2). Grayed area represents when shades were over experimental plots and white area when shades were removed. White vertical lines indicate sampling days; asterisks overlaying shade treatment light data indicates a significant reduction in seagrass above-ground biomass and percent cover relative to control for that sampling event (percent cover only for week 6 in G2); dashed lines indicate a biologically significant light threshold based on shading study results; solid black lines denote the derived management light threshold.

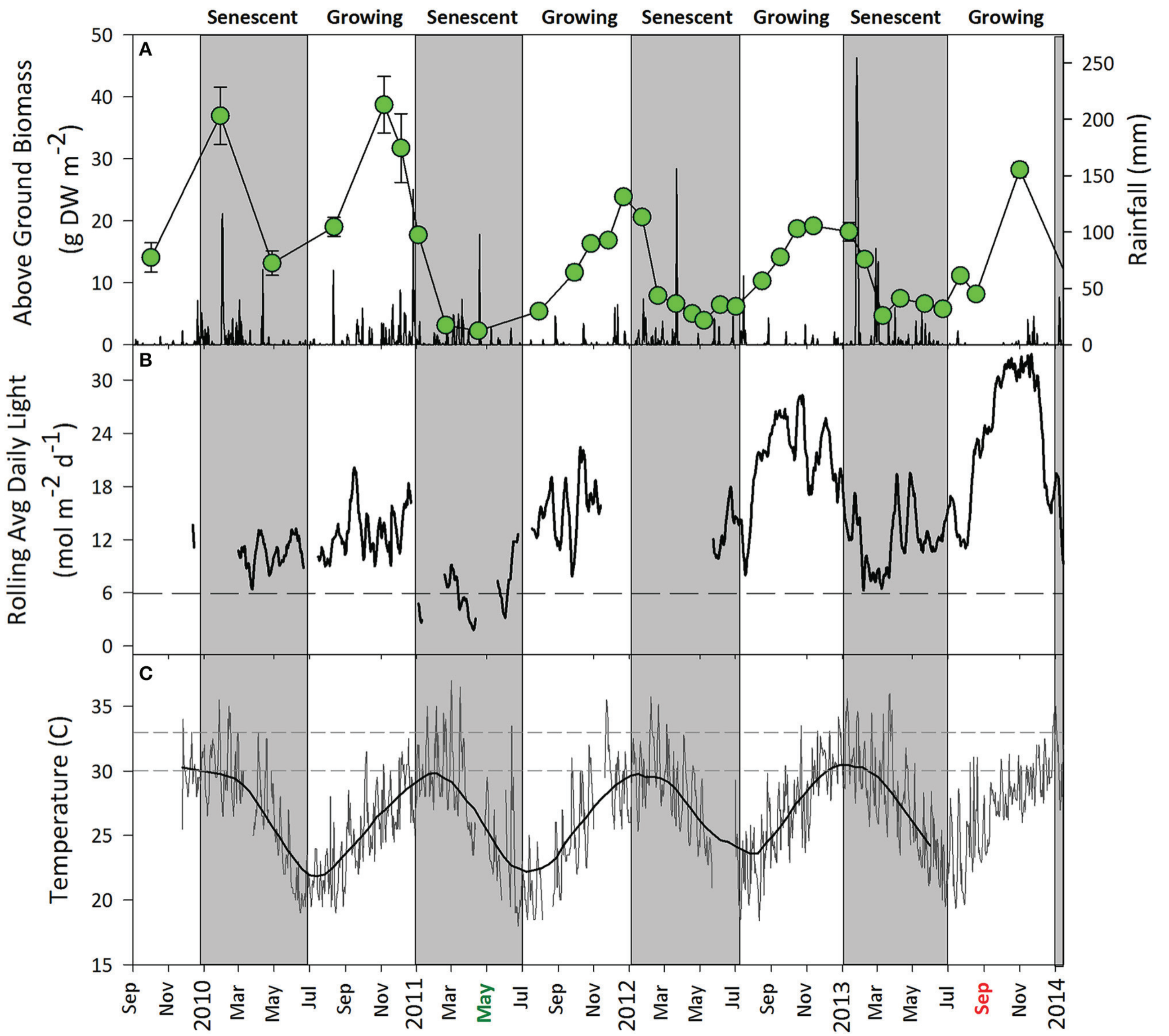

FIGURE 6 | Environmental conditions in the *Zostera muelleri* ssp. *capricorni* meadow, September 2009 to July 2013. (A) Mean ± SE seagrass percent cover (open circles) and daily rainfall. **(B)** Rolling mean total daily benthic light with trialed species- and region-specific light threshold for *Z. muelleri* ssp. *capricorni* (dashed line). **(C)** Daily maximum water temperature with critical temperature threshold for *Z. muelleri* ssp. *capricorni* (Collier et al., 2011) (dashed line). Grayed areas represent indicative senescent periods (~Jan-July) for local seagrasses; however, the onset of senescence and return to growing periods is environmentally driven rather than a fixed date. Dredging activity in Gladstone Harbour is indicated on the x-axis starting in May 2011 (green) and finishing in September 2013 (red).

only sustains seagrass cover, but also provides energy reserves to be maintained or increased when conditions are good is likely important to ensure short-term stress events do not push the plant past a point of no return.

The quality of the light environment reaching seagrasses may be as important as the quantity of light received. Dredging, for example, typically increases particulate matter in the water column which affects spectral quality (Kirk, 1994). The size and type of particles re-suspended by dredging activity alter PAR transmission in a non-linear manner, with some wavelengths being more attenuated than others, resulting in a reduced light environment with a shift toward yellow wavelengths (Kirk, 1994; Gallegos et al., 2009). Therefore, a light threshold value used for monitoring seagrass health during a dredging campaign, as determined according to the full PAR spectrum available, may overestimate the actual light available for photosynthesis as PAR measurements do not distinguish spectral shifts (Van Duin et al., 2001; Zimmerman, 2003). Light quality in Gladstone waters has explicit spatial variability, with broader spectral transmission in the outer harbor compared to the inner harbor, yet dredging had no effect on these spectral signatures when measured during the dredging campaign that occurred during this study (Chartrand et al., 2012). The region is naturally highly turbid and therefore already exhibits a yellow-enhanced light signature due to the particle load in the water column and was not further skewed with additional sediment re-suspension from the dredge operation. While a more accurate threshold applying photosynthetic usable radiation (PUR) in place of PAR could resolve any effects of wavelength-specific water column absorption we did not need to alter light threshold values to incorporate spectral shifts from dredging in this instance.

Short term repeated shading and respite (fortnightly) in the present study was carried out to mimic repeated acute attenuation events from turbidity plumes followed by subsequent "relief" intervals. In providing a 14 day period of respite after shading was applied, *Z. muelleri* ssp. *capricorni* appeared to cope for 12 weeks with even the highest shade treatment, which had significantly impacted treatment plots shaded continuously after only 6–8 weeks. A study by Biber et al. (2009) also explored extreme attenuation events interspersed with recovery periods of varying length. They found that recovery intervals at least equal to the period of light deprivation were essential for long term survival.

Other investigations into *in situ* light requirements on *Zostera* spp. agree with the measured light effects and management threshold derived in this study (Dennison and Alberte, 1985; Moore et al., 1997; Thom et al., 2008; Collier C. J. et al., 2012). Collier C. J. et al. (2012) tested reduced light conditions during laboratory shading experiments on *Z. muelleri* ssp. *capricorni* also collected from Gladstone Harbour and found shoot density declined after 8.7 weeks under 4.4 mol photons m^{-2} d^{-1} and 10.6 weeks under 9.5 mol photons m^{-2} d^{-1}. For the congeneric *Z. marina*, Dennison and Alberte (1985) found a significant reduction in *Z. marina* production rates with average daily scalar light levels of ~3.7 mol photons m^{-2} d^{-1} under shades compared to unshaded controls (8 mol photons m^{-2} d^{-1}) during critical summer growing conditions. Moore et al. (1997) found similar results where sites with high light attenuation (2.7 mol photons m^{-2} d^{-1}) over 30 days was lethal to *Z. marina* transplants compared to those with higher water clarity (13.4 mol photons m^{-2} d^{-1}). More recent work on *Z. marina* found light requirements for long-term survival is 3 mol photons m^{-2} d^{-1} and at least 7 mol photons m^{-2} d^{-1} for non-light-limiting growth conditions during critical growing months (Thom et al., 2008).

Deriving a Light Threshold for Management

Developing effective management tools and appropriate mitigation strategies to protect seagrasses from a large-scale dredging campaign requires information on the distribution, light requirements and tolerances of local seagrass communities. Shading studies and the 4-year seagrass and light monitoring program provided the means to develop an effective and ecologically-derived management threshold. A 14 day integrated daily light value was used to establish a light threshold, which if maintained, would allow sufficient light to maintain local *Z. muelleri* ssp. *capricorni* seagrass condition in Gladstone Harbour during dredging.

With no significant effects of shading on seagrass growth during either of the senescent seasons, a seagrass light management threshold was only defined for the growing season when *Z. muelleri* ssp. *capricorni* was sensitive to shading treatments. Both growing season studies clearly indicated light below 4 mol photons m^{-2} d^{-} is insufficient to maintain seagrass growth and or survival. In the second growing season study, light levels 2 weeks prior to a decline in seagrass measured between 4 and 5 mol photons m^{-2} d^{-1}, indicating morphological changes in *Z. muelleri* ssp. *capricorni* can take place in Gladstone at light intensities of $\leq$ 5 mol photons m^{-2} d^{-1}.

While the time to measurable loss in the first growing season was between 4 and 8 weeks, more frequent sampling during the second growing season documented appreciable declines in seagrass cover as early as 6 weeks under light limiting conditions. A study by Adams et al. (2015) found the timeframe over which light history and *Z. muelleri* above-ground biomass best correlated was from 8 to 35 weeks, however, they recognized management actions also should be triggered well before these measured reductions in biomass occur.

A range of bioindicators have been reviewed for use in seagrass monitoring programs to measure environmental pressures such as dredging (McMahon et al., 2013). While some metrics may be more sensitive on shorter time scales (e.g., rhizome sugars or ETR_{max}) to changes in the light climate (reviewed in McMahon et al., 2013), the ability to measure changes rapidly in relation to anthropogenic pressures (i.e., dredge operations) is important to apply an appropriate and timely management response. In the current study, above-ground abundance (either biomass or percent cover) reacted to light conditions within a timeframe that would allow a management response to be applied that could abate seagrass loss (i.e., move dredge to a new location), whereas shoot density was less sensitive to attenuated light. Other studies have also found shoot density to be a less sensitive metric; *Z. muelleri* ssp. *capricorni* alters leaf morphology before shoot loss under reduced light treatments, making above-ground biomass or cover a more sensitive indicator of change than shoot density as a consequence of environmental conditions (Rasheed, 1999; Collier C. J. et al., 2012).

As a conservative approach to protecting seagrass, a management light threshold needed to provide >5 mol photons m^{-2} d^{-1} with some degree of buffer from potential impact to the plants and to ensure the plants not only maintained physical presence, but could generate energy stores. The threshold needed to ensure protection of seagrasses from deteriorating light conditions, while also having a credible fit with natural background light variability within the local meadow. If the threshold value was set too high and therefore routinely breached without measureable impacts to seagrass condition, it would be ineffective as a management tool. Conversely, a value too low that was never measured *in situ* in spite of concurrent declines in seagrass cover would likewise be inappropriate. A light threshold of 6 mol photons m^{-2} d^{-1} was therefore used in a compliance framework by government regulators and management authorities to prevent measurable loss of seagrass from dredge related light attenuation in required management zones during dredging activity in Gladstone Harbour. This light threshold was considered in parallel with turbidity monitoring to ensure effects of turbidity related to the dredge vs. background conditions could be resolved (GPCL, 2012b). During the dredging campaign light was maintained above the management threshold for the growing season at all of the prescribed seagrass management zones (GPCL, 2012a). This coincided with the presence of the largest seagrass meadows in the greater region during and post-dredging (Carter et al., 2015) and provides confidence that the approach used could be applied elsewhere for managing seagrasses.

While much research is focused on quantifying seagrass light requirements (Dennison, 1987; Staehr and Borum, 2011; Collier et al., 2016), this work has focused on the application of seagrass light requirements for use in a management setting of a large-scale dredging program. The absolute threshold value detailed here is not as critical as the approach used to derive a light-based model for seagrasses. The successful approach developed could readily be applied in other settings with sufficient knowledge of local seagrass dynamics and light conditions.

A range of additional measures would further improve the use of light thresholds to effectively manage seagrasses during dredging and other anthropogenic activities impacting on the light environment:

1. *Combine threshold assessments with effective sub-lethal bio-indicators of light stress*-A bioindicator that responds over days rather than weeks, and prior to actual physical declines in the plant, would dramatically improve the reaction time for management decisions to adjust dredging activities before declines occur. McMahon et al. (2013) identified a range of indicators that may be useful to measure sub-lethal changes, however, most still require substantial processing time. An indicator would ideally be measured and processed within 24–48 h for effective reactive management of dredging operations. Progress toward developing molecular indicators of sub-lethal seagrass light stress provides the most promising approach (Macreadie et al., 2014).
2. *Further investigations of the effect of water temperature*-Temperature is a known driver of temperate seagrass meadow dynamics and plant metabolism (Zimmerman et al., 1989; Olesen and Sand-Jensen, 1994; Staehr and Borum, 2011). However, the role of seasonally-driven temperature fluctuations on tropical seagrasses is inadequately described (McKenzie, 1994; Rasheed and Unsworth, 2011) despite work showing temperature governs the light intensity needed for a net carbon balance (Lee et al., 2007; Collier et al., 2011). Such effects need to be studied in other species and in greater detail to understand how temperature may act as a secondary driver of seagrass light thresholds for management.
3. *Research on the impacts of whole plant dynamics on light requirements*-Recent work has implicated cascade effects of reduced light on degradation of below-ground structures and the surrounding micro-environment (Terrados et al., 1999; Borum et al., 2006; Koren et al., 2015). Compromising below-ground root/rhizome integrity has negative implications for meadow resilience and the ability to resist short-term stresses (Vonk et al., 2015). Understanding whole plant dynamics and how light reduction affects oxygen transport and below ground viability is vital to understand whether thresholds are in line with whole plant coping strategies.
4. *Modification of light requirements under cumulative long-term impacts*-Poor water quality prior to a major development may exacerbate efforts to manage additional impacts on already chronically stressed seagrass. Prolonged physiological strain from cumulative pressure over time may alter the plant's capacity to cope with further reduced light and may influence the light levels required for recovery.

CONCLUSION

This study characterized the tolerance of *Z. muelleri* ssp. *capricorni* to light attenuation on an intra- and inter-annual cycle using *in situ* shading studies and light history monitored over a 4-year period. This information was used to develop a locally-relevant management plan to protect seagrasses from dredging-related impacts to the light environment. A light threshold of 6 mol photons m^{-2} d^{-1} was successfully trialed as part of a compliance program for mitigating dredging impacts. This minimized the risk that *Z. muelleri* ssp. *capricorni*, the dominant local species, was affected by dredge turbidity plumes within prescribed management zones. When implementing a light management strategy it is critical that local conditions, species and context are considered.

AUTHOR CONTRIBUTIONS

KC, MR, CB, and PR together designed the research project. KC led the study and drafted the manuscript with the assistance of MR and AC. CB and AC provided major assistance in field execution and data analysis. All co-authors commented on and approved the final manuscript draft.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmars.2016.00106

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frontiers in Marine Science

ORIGINAL RESEARCH
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Chemical Assessment of Ballast Water Exchange Compliance: Implementation in North America and New Zealand

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Fluorescence by naturally occurring dissolved organic matter (FDOM) is a sensitive indicator of ballast water source, with high FDOM in coastal ballast water decreasing typically dramatically when replaced by oceanic seawater during ballast water exchange (BWE). In this study, FDOM was measured in 92 ships arriving at Pacific ports on the US west coast and in New Zealand, and used to assess their compliance with ballast water regulations that required 95% replacement of port water to minimize invasive species risks. Fluorescence in many ships that reported BWE was significantly higher than is usual for oceanic seawater, and in several cases, significantly higher than in other ships with similar provenance and ballast water management. Pre-exchange source port conditions represented the largest source of uncertainty in the analysis, because residual coastal FDOM when highly fluorescent can significantly influence the fluorescence signature of exchanged ballast water. A meta-analysis comparing the intensities of FDOM in un-exchanged ballast tanks with calculated pre-exchange intensities assuming that ships all correctly implemented and reported BWE revealed notable discrepancies. Thus, the incidence of high-FDOM port waters was seven times lower in reality than would be expected on the basis of these calculations. The results suggest that a significant rate of reporting errors occur due to a combination of factors that may include inadequate BWE and unintentional or deliberate misreporting of ballast water management.

Keywords: Pacific Ocean, fluorescence spectroscopy, FDOM, invasion vectors, invasive species, AIS, NIS, CDOM

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INTRODUCTION

The transfer of ballast water between ports is an effective mechanism for moving a diverse assemblage of marine and estuarine organisms around the globe, posing considerable risk to the marine environment (Carlton and Geller, 1993; Ruiz et al., 1997; Roman and Darling, 2007). In the United States, controlling ballast water discharge is viewed as an important factor in the management of bays, estuaries, and the Great Lakes (Costello et al., 2007; Bailey et al., 2011). In New Zealand, economically and socially important fisheries are threatened by large volumes of ballast water discharged each year (Hewitt and Campbell, 2007). In both countries, ballast water is the suspected vector for several marine introductions. Damage caused to the Great Lakes by the Zebra Mussel, including extensive fouling and clogging of water intake pipes and impacts on native species, led in 1993 to the first ballast water exchange (BWE) requirements for ships entering the Great Lakes from outside the US exclusive economic zone. This authority was soon extended to other regions of the country by the National Invasive Species Act of 1996 (H. R. 4283, 104 Congress of the United States).

Ballast water is carried by vessels to provide stability and trim during sailing and during loading and unloading operations. It is usually loaded at the same time that cargo is unloaded and discharged in exchange for cargo, but may also be transferred between tanks within a vessel and carried for up to several months or even years. During BWE, port water within ballast tanks is replaced with oceanic water sourced outside of the coastal zone, preferably at least 200 nautical miles (nmi) from shore, although coastal BWE is often performed along routes that remain closer to shore (Miller et al., 2011). Depending on a range of factors including the tank design, type of exchange method used, and characteristics of individual species, BWE is capable of reducing concentrations of coastal organisms by 80–95% (Gray et al., 2007; Minton et al., 2015). The effectiveness of current BWE policy at reducing invasion rates is difficult to evaluate (Costello et al., 2007) and policy efforts over more than a decade have been directed toward replacing BWE with better technological solutions (Briski et al., 2015) and concentration-based performance standards (Albert et al., 2013). However, a range of setbacks have hampered the widespread adoption of new treatment technologies and performance standards with the result that BWE is still the only ballast water treatment method in widespread use (Minton et al., 2015).

Both the United States and New Zealand governments require commercial vessels arriving from overseas to treat or exchange their ballast water before discharge to reduce the risk of releasing invasive coastal species (MAF, 2007; Miller et al., 2011; United States Coast Guard (USCG), 2012a,b). Despite the legislative requirement for BWE in both countries, it is difficult to evaluate ships' claims regarding the origin and management of ballast water. In the United States, the process for determining whether a ship has conducted BWE are detailed in the US Coast Guard's Navigation and Inspection Circular 07–04, Ch-1. Ballast water management records may be examined, and salinity readings may be taken if non-compliance is suspected. In New Zealand, the Ministry of Primary Industries Biosecurity Division prohibits the discharge of ballast water into New Zealand waters without the permission of an inspector (MAF, 2005, 2007). To obtain permission, the vessel's Master must provide a signed declaration that the ballast water was subject to mid-ocean BWE. Inspectors approve ballast water discharge based on a combination of factors including agreement between ballast management records and salinity. In both countries, ballast water with salinity between 30 and 40 is considered consistent with BWE. However, this criterion fails to reliably detect ballast water originating in Pacific rim ports, since many ports in this region have high salinities either seasonally or year-round (Doblin et al., 2010).

Previous research indicates that fluorescence by naturally occurring dissolved organic matter (FDOM) is a robust coastal tracer, with sensitivity that exceeds many other chemical tracers including salinity and trace elements (Murphy et al., 2008a, 2013; Doblin et al., 2010). FDOM quantifies the organic matter fraction that absorbs light and reemits the radiation as fluorescence (Lakowicz, 2006). In estuaries, FDOM intensities vary with salinity gradients and biological activity as well as anthropogenic factors such as industrial effluent, and agricultural and urban runoff (Coble, 1996; Stedmon and Markager, 2005; Walker et al., 2009; Guo et al., 2011). Moving offshore away from terrestrial sources and as a result of exposure to sunlight, FDOM derived from terrestrial materials decreases (Duursma, 1974; Blough and Del Vecchio, 2002; Murphy et al., 2008b; Nelson et al., 2010). Because oceanic levels of FDOM are very low relative to concentrations at the coast, it can be deduced that samples with high FDOM are of coastal origin.

Previous studies have used fluorescence excitation-emission matrix spectroscopy to identify wavelengths most appropriate for measurement (Murphy et al., 2004, 2006). These found long-wavelength fluorescence associated with terrestrial organic matter to be an effective indicator of BWE. In shipboard experiments conducted in the North Pacific and Atlantic oceans, Murphy et al. (2006) determined that a threshold of 0.7 QSE (parts per billion quinine sulfate equivalents) measured at the C3* wavelength pair ($\lambda_{ex}/\lambda_{em} = 370/494$ nm) discriminated between exchanged and unexchanged ballast water in >95% of tests ($N = 40$ ballast tanks), some of which were in the range of oceanic salinities. An extensive survey (>2000 samples) of C3* in ports and at varying distances from land confirmed that large differences in coastal vs. oceanic FDOM levels hold in the Pacific Ocean (Murphy et al., 2013). However, natural variability in coastal FDOM levels, which may legally represent as much as five percent of the water in an exchanged ballast tank, make it difficult to rely upon a simple C3* threshold. For example, assuming oceanic C3* levels of 0.5 QSE, any ship carrying ballast originally from a location where C3* exceeds 4.5 QSE will exceed 0.7 QSE even after performing 95% BWE.

In practice, given incomplete knowledge of FDOM distributions in coastal environments on a global scale, reliable chemical assessments of BWE must rely upon a forensic approach, in which multiple lines of evidence feed into the judgment of a vessel's compliance. Assuming that FDOM levels that were present in the ballast water tanks prior to BWE are unknown, then port survey data and/or data from other vessels with ballast from the same location can help to constrain estimates of the likely contribution of port water to the measured FDOM signal upon arrival. To test this approach, FDOM was measured in a diverse cohort of vessels ($N = 92$ ships) boarded by inspectors at various ports along the US west coast and New Zealand. The results were used to assess BWE compliance of individual ships and to gauge the overall level of compliance among the vessel cohort.

MATERIALS AND METHODS

Experimental Design

Replicate ballast water samples were collected from 99 ballast tanks in 92 ships arriving to the United States or New Zealand. In the United States, ballast water samples were collected from 73 vessels that arrived at ports in California (47), Oregon (10), and Washington (16) in 2008 and 2009.

Samples were collected by ballast water inspectors from three state agencies: the California State Lands Commission (CSLC), the Oregon Department of Environmental Quality (ODEQ), and the Washington Department of Fish and Wildlife (WDFW). In New Zealand, ballast water samples were collected from 19 vessels that arrived at the ports of Auckland (17), Tauranga (1), and Taharoa (1) in May, 2010. Sampling was performed by Ministry of Primary Industries (MPI, formerly Ministry of Agriculture and Forestry MAF) biosecurity inspectors, assisted by one researcher. Vessels of a range of types and trading histories were selected in an effort to maximize sample diversity. Ballast water source and management was self-reported by the vessel.

Sampling

Similar sampling methodologies were implemented in the United States and in New Zealand. Ballast water samples were collected through an open manhole from a single tank per vessel in the United States and one or two tanks per vessel in New Zealand. Three replicate samples were collected using large Clear-View™ PVC bailers (45.72×2.54 cm, 342 mL) from the vertical midpoint of the accessible sampling depth. The bailers have a stopper ball which allows them to collect samples from select depths. Water flows through the tube as the bailer is lowered into the tank, then when the bailer is retrieved the stopper-ball drops to the bottom of the tube sealing it. Once filled, the bailers were drained into a 60 mL syringe then filtered using Whatman 0.45 μm PVDF syringe filters into pre-ashed 125 mL amber glass bottles. All equipment was subject to stringent cleaning prior to sampling, bailers and syringes, and filters were acid washed (10% HCl) and rinsed with 18 MΩ deionized water and air dried in a laminar flow hood. Salinity was measured using a hand-held refractometer.

For all tanks scheduled for discharge, data regarding ballast water sources and management were obtained from ballast water reporting forms, which constitute legal declarations to the National Ballast Water Information Clearinghouse in the US and to MPI Biosecurity in New Zealand. For those tanks that were not to be discharged in the sampling port, source and management data were collected from the vessel's log books by the ballast water inspector. On the basis of these reports, each sampled tank was assigned to one of four management categories: exchanged in mid-ocean >200 nmi from shore (BWE, $n = 57$), exchanged <200 nmi from shore (BWEc, $n = 19$), filled from empty in the mid-ocean (FS, $n = 11$), or carrying unexchanged port water (none, $n = 12$).

Laboratory Analyses

FDOM fluorescence was measured using a benchtop Fluorolog®-3 spectrofluorometer (Horiba Jobin Yvon, Edison, NJ). Undiluted filtered seawater samples were analyzed in ratio mode using a 0.5 s integration time and a 1-cm quartz cell held at 20°C. Fluorometer bandpasses were set to 5 nm for both the excitation and emission monochromators. The Fluorolog-3 is configured with a single excitation monochromator (1200 grooves/mm) blazed at 330 nm and a dual emission monochromator (1200 grooves/mm) blazed at 500 nm, a water-cooled, red sensitive photomultiplier tube and a 450-watt Xenon arc lamp.

Data were corrected for instrumental and lamp variability and normalized to quinine sulfate fluorescence intensity as previously described (Murphy et al., 2010). Fluorescence can be suppressed by absorbing species in the sample matrix, in a phenomenon known as the inner-filter effect (IFE). Suppression is below 5% at wavelengths where total absorbance (A) is below 0.042 in a 1-cm cell (Kothawala et al., 2013). Absorbance at 370 nm measured using a Cary 4E UV–Visible spectrophotometer was always below 0.015 m^{-1} so no inner filter correction was necessary. Fluorescence intensities were calibrated against a quinine sulfate dilution series and are expressed in units of concentration (ppb quinine sulfate equivalents, QSE). An approximate conversion of these data to Raman Units (RU, normalized to the area of the Raman peak in a clean water blank excited at 350 nm) is obtained by dividing intensities in QSE by 100 (Murphy et al., 2010). Data are reported here for a single wavelength pair, C3* ($\lambda_{ex}/\lambda_{em} = 370/494$ nm) that has been extensively studied in the context of BWE, and for which BWE thresholds have already been developed and tested (Murphy et al., 2006, 2013; Doblin et al., 2010).

Chemical Assessments of Compliance

Since terrestrially derived FDOM in the open surface Pacific Ocean far from land is low and relatively stable compared to at the coasts (Nelson et al., 2010), then a lower bound for C3* prior to BWE can be deduced from measured C3* following BWE (Equation 1)

$$C3^*_{pre\ BWE} = \frac{C3^*_{post\ BWE} - \varepsilon * C3^*_{ambient}}{(1-\varepsilon)}$$

In Equation (1), $C3^*_{post\ BWE}$ is the measured fluorescence intensity in a ballast tank was reported as having undergone BWE, $C3^*_{pre\ BWE}$ is the calculated fluorescence intensity prior to BWE, and ε is the BWE efficiency. $C3^*_{ambient}$ is the fluorescence intensity in the ambient ocean where BWE was performed.

In the calculations, BWE efficiency (ε) was assumed equal to the minimum level specified by law (95%), except in the case of ballast tanks filled from empty in the ocean (FS). For these a higher exchange efficiency (99%) was assumed based on earlier studies (Cohen, 1998; Drake et al., 2007). Filling at sea is relatively efficient because the only sources of port signals are residual volumes of unpumpable ballast water and sediments. $C3^*_{ambient}$ was assumed equal to 0.5 QSE in the open ocean, and =1 QSE in coastal exchange zones. These levels are consistent with surveys in the North Pacific (Murphy et al., 2013) and are probably conservative (i.e., represent upper limits) except when BWE was performed north of 45°N where oceanic CDOM is relatively elevated (Nelson et al., 2010). If FDOM at the site of BWE was actually higher than the assumed level, this would result in $C3^*_{pre\ BWE}$ being slightly overestimated, of if lower then $C3^*_{pre\ BWE}$ would be slightly underestimated. However, a large over- or under-estimation is unlikely because even a 50% error in the assumed oceanic C3* represents no more than a small absolute difference in post-exchange C3*. Conversely, $C3^*_{pre\ BWE}$ is very sensitive to BWE efficiency since a decrease from 95% to 90% efficiency doubles the influence of the residual port signal.

Calculated $C3^*_{pre\ BWE}$ was used in two ways to assess compliance by individual vessels. First it was compared with measured C3* at the port of origin, when port data were available from earlier surveys and published reports. Second, it was used in comparisons with measured C3* in other ships that loaded ballast water in the same location at approximately the same time (within 2 weeks). To assess compliance by the cohort as a whole, the distribution of calculated $C3^*_{pre\ BWE}$ was compared with the measured distribution of C3* in ballast tanks that were reported as having not undergone BWE ($n = 48$). The sample size for this comparison was increased by including data from any randomly-sampled tank containing unexchanged ballast water in our databases ($n = 36$). To avoid biasing the results, ships in our database that were deliberately targeted on the basis of source characteristics were excluded from this comparison.

RESULTS

Table 1 summarizes C3* fluorescence and salinity measurements for each sampled tank, classified by ballast water source and reported ballast water management ($N = 99$ tanks from 92 ships). The majority of tanks (88%) reportedly underwent some type of ballast water management. Most were exchanged in mid-ocean more than 200 nmi from land (57%) or in coastal waters (20%), and 11% were filled from empty at sea. All ballast tanks reportedly sourced or exchanged at least 200 nmi from land (BWE and FS categories) had salinities between 31 and 41, i.e., within the range of salinities considered by regulatory agencies to be consistent with oceanic sources.

Figure 1 shows the distribution of fluorescence intensities among tanks sampled in each management category. Intensities are shown as multiples of the BWE threshold, t_c. As expected in ships that reported no BWE, C3* always exceeded t_c, while in half of the tanks, t_c was exceeded by more than five times. Conversely, fluorescence intensities in exchanged ballast tanks were frequently much higher than expected. Among tanks that reportedly underwent mid ocean BWE or were filled at sea (BWE and FS, respectively), 54% of tanks had C3* fluorescence exceeding t_c and 25% of tanks had fluorescence exceeding $3t_c$. Among 19 tanks that reportedly underwent coastal exchange (BWEc), 36% exceeded $3t_c$, and 26% exceeded $4t_c$.

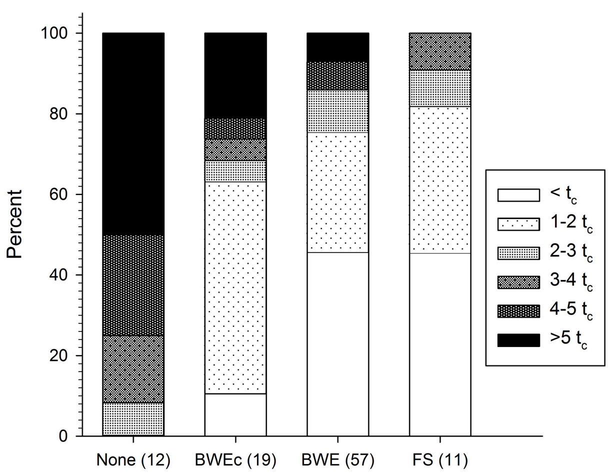

FIGURE 1 | Measured C3* fluorescence in 99 ballast tanks as a function of reported management category. Fluorescence is expressed as a multiple of the BWE threshold ($t_C = 0.7$ QSE) proposed by Murphy et al. (2006). Management categories are unexchanged (none), coastal exchange (BWEc), mid-ocean exchange (BWE), and filled at sea (FS), with number of tanks in each category listed in parentheses.

In **Figure 2**, fluorescence intensities measured in ships' ballast are mapped according to the reported geographical source of the ballast water. For unexchanged ballast water, the reported source was in a port, and for exchanged ballast water, the reported source was the offshore location where BWE took place. Blue symbols indicate low fluorescence consistent with oceanic sources, and orange and red symbols indicate high fluorescence consistent with coastal sources. C3* fluorescence was typically highest in tanks ballasted near land and lowest in ships that reported oceanic BWE. However, a significant number of tanks that were reportedly exchanged in the open ocean far from land stand out as obvious exceptions to this rule.

Table 1 contains the measured and reported data for each sampled ballast tank. Additionally, the final column contains calculated source intensities for reportedly exchanged ballast tanks, i.e. estimates of C3* prior to BWE deduced using Equation (1), assuming BWE was performed properly. These data are used in **Figure 3** to compare the distribution of calculated source intensities with the measured distribution of source intensities in unexchanged ballast tanks. **Table 1** shows that many calculated source intensities (Cases 3, 19, 21-23, 27, 32, 38, 46, 56, 58, 60–64, 83, 89, 97) represent extreme outliers. Most would remain outliers if the assumptions of the calculation were relaxed by assuming that C3* at the exchange location had been 50% higher and BWE efficiency were below 85%. Overall, these data suggest that in many cases BWE was either misreported or undertaken with much less than the mandated 95% exchange efficiency.

A number of ships in this survey originated from ports that have previously been surveyed by our group. These port survey data can be used to explore whether high C3* might reasonably be explained by residual (<5%) quantities of port water. Cases 3 and 4 represent two ballast tanks on the same ship ballasted in the port of Melbourne and later reportedly exchanged. Port surveys of FDOM in Melbourne do not support this reporting: C3* in both tanks (1.4 and 3.2 QSE) was within the typical range measured at the port of Melbourne during winter and spring surveys in 2007 whereas calculated pre-BWE C3* (9.6 and 54.4 QSE) greatly exceeded this range (Doblin et al., 2010). Similarly, Cases 57–67 represent ships that reportedly filled empty tanks in the Pacific Ocean at least 200 nmi from land, where C3* should have been extremely low. However, measured C3* intensities are consistent with predominantly open ocean sources in only two cases (57 and 65, with C3* $\leq$0.55). In six other cases, C3* intensities were in the range of 1.3–3.1 QSE, suggesting a moderate to large contribution by residual port water. Seasonal surveys at Los Angeles port and coastal waters in California have been conducted over several years by our group and indicate low background C3* in the port (<2–3 QSE, Murphy et al., 2009)

TABLE 1 | Mean fluorescence intensities ($C3^* = 370/494$ nm) measured in randomly sampled ballast tanks in ships arriving to Pacific Ocean ports in this study.

Case	Date	Age (days)	Source region	Source location	Management	$C3^*$ measured		Salinity	$C3^*_{pre\ BWE}$
						mean	*SD*		(calculated)
1	20/05/2009	11	Africa	Durban	BWE	0.84	0.0	35	7.4
2	12/05/2010	5	Australia	Melbourne	BWE	0.53	0.1	37	1.2
3	14/05/2010	4	Australia	Melbourne	BWE	3.19	0.2	36	54.4
4	15/05/2010	0	Australia	Melbourne	BWEc	1.43	0.1	37	19.1
5	15/05/2010	0	Australia	Melbourne	BWEc	0.85	0.1	37	7.5
6	6/05/2010	1	Australia	Sydney	BWE	0.58	0.2	41	2.1
7	20/05/2010	2	Australia	Sydney	BWE	0.38	0.0	36	0.5
8	23/10/2008	16	Caribbean	Coast (<2nmi)	none	1.81	0.1	35	
9	20/05/2009	12	China, N. East	Lianyungang	BWE	0.56	0.0	35	1.8
10	10/05/2010	12	China, N. East	Qingdao	BWE	0.59	0.1	35	2.2
11	27/01/2009	8	China, N. East	Longkou	BWE	1.68	0.0	40	24.2
12	12/03/2009	20	China, N. East	Longkou	BWE	0.95	0.0	37	9.5
13	25/06/2009	12	China, N. East	Dalian	BWE	0.93	0.0	35	9.0
14	4/12/2008	10	China, N. East	Tianjin	BWEc	1.59	1.2	34	12.9
15	17/05/2010	9	China, South	Singapore	BWEc	1.10	0.2	32	3.1
16	3/11/2008	7	China, S. East	Yantian	BWE	0.36	0.0	35	0.5
17	15/04/2009	15	China, S. East	Yantian	BWE	0.75	0.1	36	5.5
18	12/05/2010	25	China, S. East	Wenchong	none	22.90	1.8	4	
19	12/05/2010	8	China, S. East	Wenchong	BWE	3.67	0.5	34	63.9
20	15/01/2009		China, S. East	Zhanjiang	none	3.87	0.5	33	
21	12/05/2009	12	China, Yangtze	26nmi from Shanghai	BWE	3.75	0.1	31	65.6
22	14/05/2010	10	China, Yangtze	Shanghai	BWEc	6.49	0.1	25	110.8
23	*14/05/2010*	*36*	China, Yangtze	*Shanghai*	*BWEc*	*3.94*	*0.3*	*nd*	59.7
24	13/11/2008	12	China, Yangtze	Shanghai	BWE	1.49	0.5	33	20.3
25	29/05/2009	14	China, Yangtze	Shanghai	BWE	1.15	0.1	34	13.5
26	30/10/2008	206	China, Yangtze	Kouan Shipyard, Taizhou	none	16.69	0.2	0	
27	20/05/2009	25	China, Yangtze	Changshu	BWE	3.53	0.4	32	61.2
28	28/05/2009	16	China, Yangtze	Nantong	BWE	1.07	0.2	35	11.9
29	16/10/2008	4	Germany	Bremerhaven	BWE	0.49	0.0	32	0.4
30	5/12/2008	6	South America	Purto Quetzal, Acajutla	BWE	0.52	0.0	36	0.9
31	14/05/2009	11	Indonesia	Jakarta	BWE	0.43	0.1	36	0.5
32	6/05/2010	16	Indonesia	Surabaya	BWEc	4.02	0.3	40	61.4
33	20/11/2008	5	Indonesia	Tanjungbalai	BWE	0.61	0.0	37	2.7
34	18/01/2009	7	Japan	Chiba	BWE	0.51	0.2	34	0.7
35	7/11/2008	19	Japan	Chiba	BWE	1.66	0.2	36	23.7
36	22/07/2009	8	Japan	Chiba	BWE	1.04	0.2	31	11.3
37	14/07/2009	9	Japan	Hachinohe	BWE	0.93	0.1	34	9.1
38	26/11/2008	16	Japan	Harima	BWE	3.08	0.4	35	52.1
39	14/05/2009	12	Japan	Kashima	BWE	0.64	0.1	35	3.4
40	29/05/2009	10	Japan	Kashima	BWE	0.64	0.1	35	3.2
41	30/04/2009	9	Japan	Kawasaki	BWE	0.52	0.0	35	0.9
42	11/07/2009	9	Japan	Otaru	BWE	0.97	0.1	31	9.9
43	2/12/2008	22	Japan	Otaru	BWE	0.89	0.1	36	8.3
44	12/11/2008	59	Japan	South Japan	BWE	1.72	0.1	35	24.8
45	7/07/2009	19	Japan	Tokyo	BWE	1.33		36	17.1
46	12/01/2009	39	Japan	Tsuneishi	BWE	2.98	0.2	35	50.2
47	23/06/2009	13	Korea	Boryeong	BWE	1.04	0.3	34	11.4
48	21/05/2009	19	Korea	Busan	BWE	0.70	0.1	33	4.4
49	4/11/2008	6	Korea	Pusan	BWE	0.98	0.1	33	10.2

(Continued)

TABLE 1 | Continued

Case	Date	Age (days)	Source region	Source location	Management	C3* measured		Salinity	C3*$_{pre\ BWE}$
						mean	*SD*		(calculated)
50	14/04/2009	18	Mexico	<100nmi	FS	0.69	0.1	36	10.8
51	18/11/2008	6	Mexico	Guaymas	BWEc	0.52	0.0	32	0.8
52	3/11/2008	4	Mexico	Manzanilla	BWE	0.67	0.0	35	3.9
53	19/11/2008	3	Mexico	Manzanilla	none	2.73	0.0	35	
54	9/12/2008	3	Mexico	Rosarito	BWEc	0.80	0.1	36	0.8
55	18/11/2008	18	Mexico	Valparaiso	BWE	0.99	0.1	33	10.3
56	13/11/2008	62	Ocean	Mid-Atlantic	BWE	6.84	0.1	37	127.4
57	8/04/2009	1	Ocean	Pacific, North	FS	0.55	0.1	32	3.8
58	8/10/2008	12	Ocean	Pacific, North	FS	2.13	0.1	*nd*	82.7
59	23/10/2008	14	Ocean	Pacific, North	FS	0.91	0.0	35	21.7
60	10/02/2009	35	Ocean	Pacific, North	FS	1.43	0.1	34	47.9
61	6/11/2008	6	Ocean	Pacific, North	FS	1.30	0.1	32	41.4
62	21/05/2010	10	Ocean	Pacific, South	BWE	3.09	0.1	35	52.3
63	14/05/2010	14	Ocean	Pacific, South	FS	2.23	0.0	38	87.8
64	20/05/2010	51	Ocean	Pacific, South	FS	1.28	0.1	36	40.4
65	17/05/2010	22	Ocean	Tasman Sea	FS	0.52	0.1	35	2.2
66	20/05/2010	18	Ocean	Pacific, South	BWE	1.64	0.1	35	23.3
67	11/05/2010	84	Ocean	Pacific, South	BWE	0.72	0.0	39	4.9
68	6/05/2010	3	Pacific Islands	Apia, Samoa	BWE	0.31	0.0	34	0.5
69	8/05/2010	34	Pacific Islands	Papeete, Tahiti	BWE	0.48	0.1	36	0.1
70	12/05/2010	42	Pacific Islands	Port Vila, Vanuatu	BWE	1.75	0.1	38	25.5
71	20/05/2010	2	Pacific Islands	Suva, Fiji	BWE	0.64	0.1	35	3.4
72	21/05/2010	111	Pacific Islands	Suva, Fiji	BWE	0.64	0.0	40	3.3
73	18/05/2010	15	South America	Balboa, Panama	BWE	0.60	0.0	36	2.6
74	24/09/2008	4	Taiwan	Kaohsiung	BWE	0.55	0.0	35	1.5
75	4/11/2008	5	Taiwan	Kaohsiung	BWE	1.17	0.0	38	13.9
76	18/05/2010	5	Tonga	Nuku'alofa	BWE	0.58	0.0	35	2.1
77			Unknown	Unknown	none	6.07	0.1	35	
78	6/08/2008	15	US—Hawaii	Hawaii	BWE	0.32	0.1	35	0.5
79	21/05/2009	63	US East Coast	NJ + East China Sea	none	2.15	0.1	36	
80	12/11/2008	11	US West Coast	Nikiski, AK	BWEc	0.89	0.0	33	0.8
81	18/11/2008	6	US West Coast	Cherry Point, CA	BWEc	0.45	0.1	32	0.8
82	20/11/2008	4	US West Coast	Los Angeles	BWEc	1.42	0.1	35	9.4
83	18/05/2009	1	US West Coast	Los Angeles	BWEc	4.05	0.1	30	62.0
84	15/04/2009	2	US West Coast	Los Angeles	BWEc	1.04	0.1	39	1.8
85	18/05/2009	7	US West Coast	Los Angeles	BWE	0.90	0.1	33	8.5
86	6/11/2008	7	US West Coast	Los Angeles	FS	0.50	0.0	35	1.4
87	12/11/2008	6	US West Coast	Mix CA ports/coast	BWE	0.49	0.0	32	0.3
88	15/04/2009	8	US West Coast	Oakland, CA	none	5.49	0.2	32	
89	8/04/2009	1	US West Coast	Oakland, CA	BWEc	3.37	0.2	35	48.3
90	13/04/2009	10	US West Coast	San Pedro, CA	BWE	0.86	0.1	35	7.6
91	4/03/2009	0	US West Coast	HI, OR	none	3.40	0.7	16	
92	10/02/2009	172	US West Coast	<50nmi	FS	0.47	0.0	42	0.5
93	7/01/2009	7	US West Coast	Portland, OR	BWEc	0.91	0.0	36	1.0
94	22/05/2009	4	US West Coast	Willbridge, OR	BWEc	1.24	0.0	35	5.8
95	21/05/2009	25	US West Coast	Seattle, WA	none	3.24	0.2	28	
96	27/05/2009	8	US West Coast	Seattle, WA	none	2.96	0.4	30	
97	21/11/2008	3	US West Coast	Seattle, WA	BWEc	2.79	0.1	32	36.8
98	13/05/2009	5	US West Coast	Seattle, WA	BWEc	1.35	0.0	33	8.0
99	16/12/2008	64	US West Coast	Vancouver, BC	none	3.75	0.0	30	

The number of days between loading and sampling of ballast water is indicated in the column "Age". Ballast water management is categorized as mid-ocean exchange (BWE), coastal exchange (BWEc), filled at sea (FS), or unexchanged (none). The final column contains calculated fluorescence prior to BWE (see main text). Missing data is shown as "nd."

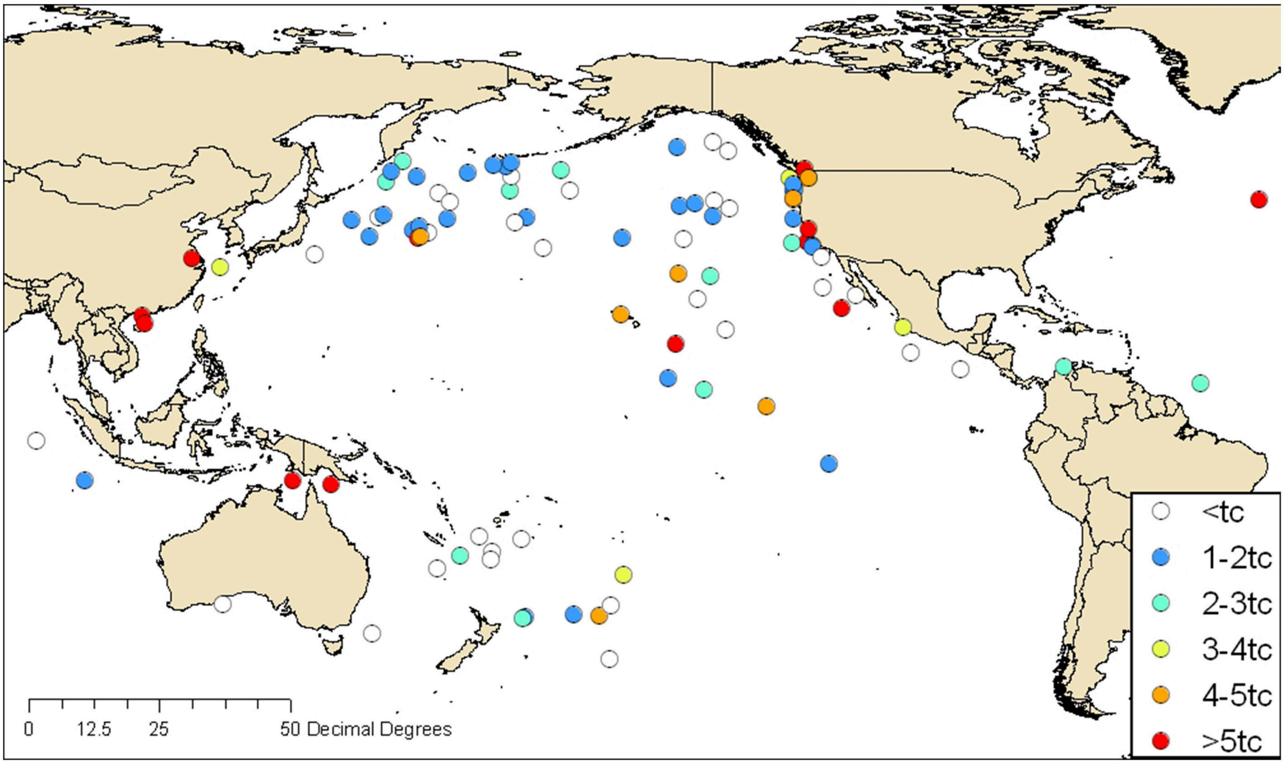

FIGURE 2 | FDOM fluorescence intensities (C3* in QSE) in ships' ballast water mapped according to the reported origin of ballast water. Symbols indicate intensities in multiples of the BWE threshold (0.7 QSE) developed by Murphy et al. (2006). Orange and red symbols indicate C3* intensities that exceed the threshold by more than four and five times, respectively.

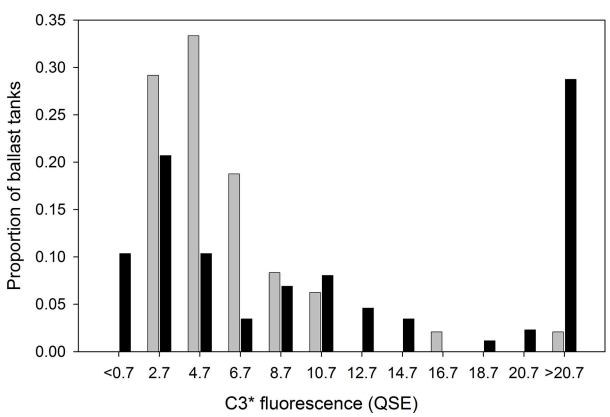

FIGURE 3 | C3* distribution in randomly sampled unexchanged ballast tanks in this study (n = 48, dark bars) compared with expected pre-BWE C3* if ballast water exchange were reported correctly (n = 79, light bars).

decreasing to below 0.8 QSE in the coastal ocean at distances exceeding 50 nmi from shore (Murphy et al., 2013). In Case 83, C3* exceeded 4 QSE after reported 95% coastal BWEc, which would require that C3* prior to BWE was around 30 times higher than the highest values measured during these earlier surveys.

The C3* measurements in **Table 1** are organized geographically to facilitate comparisons between tanks having similar ballast water sources. When two ships ballast in the same port at around the same time and undertake similar ballast water management, C3* intensities in both ships should be comparable. For example, cases 95 and 96 represent unexchanged ballast water obtained in Seattle by two different ships within a 3 week period and differ by <10%. Returning to Cases 3 and 4, these can be compared with Case 2, on another ship that ballasted in the port of Melbourne a few days earlier. For Case 2, C3* after BWE was below t_c as expected, and 3–6 times lower than in Cases 3 and 4. These results again suggest that BWE was undertaken in Case 2, but not in Cases 3 and 4. Similarly, Cases 74 and 75 from Kaohsiung are inconsistent because (1) despite tanks having been loaded and exchanged at nearby locations within a month of one another, C3* was two-fold higher in Case 75, and (2) whereas for Case 74 the estimated pre-BWE C3* is within the known range of Kaohsiung port (1–2 QSE, Murphy et al., 2009), for Case 75 it is a factor of two higher. Finally, Cases 95 and 96 with unexchanged Seattle water provide some support for the claim that BWE was attempted in Case 98, although it appears to have been much less than 95% efficient.

In most cases where fluorescence data were at odds with BWE reporting in this study, there was no evidence of irregularities in the ship's paperwork. However, the vessel involved in Cases

3 and 4 had serious enough paperwork irregularities that the port authority involved denied permission to discharge ballast water. Although our data were not the basis of this decision, the fluorescence measurements independently corroborated the inspector's suspicions regarding the integrity of the ship's records. Cases 16 and 83 also had inconsistent reporting and elevated fluorescence results.

An evaluation of reporting by the entire cohort is provided by **Figure 3**. Here, the distribution of calculated $C3^*_{pre\ BWE}$ ($n = 72$) can be compared directly with the measured distribution of C3* in ships that did not report exchanging ballast water ($n = 48$). The calculated C3* distribution has higher proportions of vessels in both the extremely low (<0.7 QSE) and extremely high (>20.7 QSE) fluorescence ranges. The low anomaly indicates that at least 10% of ships who reported BWE encountered C3* levels in the ocean lower than those that were assumed in the calculations. The high anomaly indicates that the incidence of high-FDOM ports should be around an order of magnitude higher than it actually is, if ships were all correctly implementing and reporting BWE.

DISCUSSION

This study presents the first report of dissolved organic matter fluorescence intensities ($C3^* = 370/494$ nm) in ballast tanks of randomly-sampled ships arriving to Pacific ports. It was attempted to use these data to verify BWE when reportedly undertaken for those tanks, based upon reconciling fluorescence measurements with ships' reports without direct information regarding the chemical signatures of the ballast tanks prior to BWE. Previous research indicates that fluorescence is a stable and sensitive tracer of BWE in controlled experiments for which the source waters and treatments applied are able to be carefully monitored (Murphy et al., 2004, 2006). However, in a regulatory setting these data are usually unavailable or supplied by the ship and of unknown accuracy. Applying fluorescence as tool to verify BWE in a regulatory setting therefore introduces additional practical and technical challenges.

Applying a unilateral fluorescence threshold for determining BWE compliance, e.g., $C3^* < 0.7$ QSE, would be expected to fail in two main situations. First, if a ship ballasts in a clear-water port with little terrestrial input of organic materials, then fluorescence intensities may be low regardless of whether BWE takes place. According to **Figure 3**, ports with $C3^* < 1$ QSE account for <10% of cases in our dataset. Also, tanks sampled in this study were nearly all ballasted and exchanged in the Pacific Ocean which experiences low coastal influences compared to the Atlantic Ocean (Opsahl and Benner, 1997; Siegel et al., 2002). Low-CDOM ports are therefore likely to be less common in the Atlantic Ocean. Second, verification could fail if a ship ballasts in a humic-rich port and retains 5% of this water following BWE, since residual port water could significantly elevate the total ballast water signal. Assuming BWE were performed with 95% efficiency in the mid-ocean where C3* is around 0.5 QSE, then ships that originally ballasted in ports where $C3^* > 10$ QSE would have C3* above 1 QSE. Relatively high-CDOM ports with $C3^* > 10$ QSE were uncommon in our dataset (<10% of measured tanks), although would presumably be more common had ships originated from Atlantic ports. To limit the loss of sensitivity that inevitably would result from a one-size-fits-all BWE threshold, a forensic approach considering multiple lines of evidence was employed in this study.

The chemical signature of exchanged ballast tanks was shown to be very sensitive to ballast exchange efficiency. Previous research indicates that BWE efficiencies vary by ship type and according to the method of exchange. Using the empty-refill method, exchange efficiencies exceeding 98% are typical, however, flow-through exchange allows mixing between the incoming and outgoing water and often results in exchange efficiencies well below the mandated level. Increasing BWE efficiency from 95 to 98% decreases the port signal by more than half, whereas decreasing BWE efficiency from 95 to 90% doubles it. At the same time, biological risk is similarly sensitive to exchange efficiency. If the presence of 5% coastal organisms in ballast water represents the upper limit of acceptable risk, then accepting BWE with 90% efficiency results in twice the acceptable risk, and 85% BWE triples it.

The strength of the pre-BWE signal is also critical for determining the chemical profile of an exchanged ballast tank, even when oceanic water becomes 20 times more abundant than coastal water following BWE. Thus, for moderately fluorescent ports with $C3^* = 5$ QSE, a two-fold increase in pre-BWE C3* has a similar effect on the post-exchange signal as a two-fold increase in open ocean C3*. Accurately estimating the pre-BWE signal for individual ships is difficult, since the water quality conditions encountered by individual ships while ballasting in port are subject to a number of sources of uncertainty, including temporally and spatially variable processes affecting terrestrial inputs (Stedmon et al., 2006; Yamashita et al., 2008). The picture is further complicated in ships that top up or transfer ballast water between tanks, which produces a blended chemical profile of indeterminable origin. For these reasons, it is difficult to conclusively identify ships that misreport BWE except in relatively extreme cases or when directly comparable measurements happen to be available. Approximately 10% of ships fell into this category in this study, although due to the generally conservative assumptions used in calculations together with the high prevalence of relatively low FDOM ports along the Pacific Rim (Murphy et al., 2009; Doblin et al., 2010), this probably represents a lower limit of BWE reporting/implementation errors.

Whereas conclusively determining BWE compliance by specific ships is often difficult, a meta-analysis of the chemical data is consistent with the finding that 95% BWE is not being performed as frequently as ships report. If this were not the case, then the distribution of measured C3* in unexchanged ballast tanks (**Figure 3**) should largely overlap with the pre-BWE C3* distribution back-calculated from C3* measured in exchanged ballast tanks. Instead, high-CDOM ($C3^* > 15$ QSE) source ports were at least seven times more common in the calculated vs. measured pre-BWE datasets. Overall, the results suggest that a significant rate of reporting errors occur due to a combination of factors, including inadequate BWE and unintentional or deliberate misreporting of ballast water management.

Experience from the Great Lakes of North America suggests that compliance by ships with BWE legislation is strongly linked to inspection effort (Bailey et al., 2011). Whereas, our earlier research established the scientific basis for using fluorescence spectroscopy to trace ballast water origin, this is the first study to move this technique to the level of implementation and demonstrate how the technology works when implemented by governmental inspectors. *In-situ* FDOM sensors have recently entered the market and offer the possibility of simple real-time measurements as long as instrument reliability, stability, and calibration issues are appropriately handled. Incorporating such measurements into inspection programs at Pacific rim ports could improve the detection of high-risk ballast water and the overall implementation of BWE in the region.

AUTHOR CONTRIBUTIONS

GR, MN, and KM conceived of the overall study and experimental design. MN performed the field trials and acquired the data in this study with assistance from others as described in the Acknowledgements. Statistical analyses were performed by MN and KM. KM and MN drafted the article and all authors revised it for intellectual content. All authors approve of the final version and are accountable for its accuracy.

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Assessing the status in an integrative way

frontiers in
MARINE SCIENCE

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Tales from a thousand and one ways to integrate marine ecosystem components when assessing the environmental status

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Assessing the environmental status of marine ecosystems is useful when communicating key messages to policymakers or the society, reducing the complex information of the multiple ecosystem and biodiversity components and their important spatial and temporal variability into manageable units. Taking into account the ecosystem components to be addressed (e.g., biological, chemical, physical), the numerous biodiversity elements to be assessed (e.g., from microbes to sea mammals), the different indicators needed to be studied (e.g., in Europe, 56 indicators of status have been selected), and the different assessment scales to be undertaken (e.g., from local to regional sea scale), some criteria to define spatial scales and some guidance on aggregating and integrating information is needed. We have reviewed, from ecological and management perspectives, the approaches for aggregating and integrating currently available for marine status assessment in Europe and other regions of the world. Advantages and shortcomings of the different alternatives are highlighted. We provide some guidance on the steps toward defining rules for aggregation and integration of information at multiple levels of ecosystem organization, providing recommendations on when using specific rules in the assessment. A main conclusion is that any integration principle used should be ecologically-relevant, transparent and well documented, in order to make it comparable across different geographic regions.

Keywords: ecosystems, marine, indicators, Marine Strategy Framework Directive, descriptors, criteria, assessment, integration

INTRODUCTION

The requirement to assess the environmental status of marine waters is growing across continents (Borja et al., 2008). It is also one of the challenging tasks to be accomplished in Europe, within the Marine Strategy Framework Directive (MSFD) (European Commission, 2008). The different legislative mandates to asses status coming from the MSFD, Water Framework Directive (WFD) (2000/60/EC) and Habitats Directive (92/43/EEC) and other international initiatives have produced numerous methodologies that can be applied to different ecosystem components, such as various taxonomic or functional groups, habitats, traits, physical features, or to the whole ecosystem (Birk et al., 2012; Halpern et al., 2012). Despite this wealth of methods, determining environmental status and assessing marine ecosystems health in an integrative way is still one of the grand challenges in marine ecosystems ecology research and management (Borja, 2014).

Different attempts to understand, define and assess ecosystem health have been made in recent years (Costanza and Mageau, 1999; Ulanowicz, 2000; Mee et al., 2008; Ojaveer and Eero, 2011; Borja et al., 2013; Tett et al., 2013). The concept of "good environmental status" (GEnS) integrates physical, chemical and biological aspects, together with the services provided by ecosystems, including a sustainable use of the marine resources by society (Borja, 2014). However, synthesizing these aspects into a single value will never appropriately reflect all aspects considered to derive the value (Purvis and Hector, 2000; Derous et al., 2007). Still, this step is useful when communicating key messages to policymakers or the society, reducing the complex information of the multiple ecosystem components and their important spatial and temporal variability into manageable units, which can be used in ecosystem management. Following the recommendation from Mee et al. (2008), we use the GEnS acronym because the meaning of "environmental," within the MSFD, and "ecological" (good ecological status), within the WFD, is different (see Borja et al., 2010, for differences between both concepts), implying a different emphasis between these two major pieces of legislation.

In the case of the MSFD, an appropriate integration process might be even more complex, since the assessment of the status is based upon 11 qualitative descriptors (i.e., D1: biological diversity; D2: non-indigenous species; D3: exploited fish and shellfish; D4: food webs; D5: human-induced eutrophication; D6: seafloor integrity; D7: hydrographical condition; D8: contaminants; D9: contaminants in fish and seafood; D10: litter; and D11: energy and noise), which are further divided into 29 criteria and 56 indicators of health (European Commission, 2010). An overview of MSFD descriptors, criteria and indicators is shown in **Table 1**.

The aim of this work is to present an overview of the different methods currently available to synthesize the ecosystem complexity, by aggregating and integrating information when assessing the status, focusing mostly on the descriptors related to biodiversity, namely D1, D2, D4, D6 (Cardoso et al., 2010; Prins et al., 2014). This overview would assist managers, through the guidelines provided, in taking decisions for a better management of the marine ecosystems.

ECOSYSTEM COMPONENTS COMBINATION REQUIREMENTS IN ASSESSING THE STATUS

There are different methods that can be applied to combine indicators and criteria within descriptors and across descriptors to eventually result in an assessment of GEnS for a specific geographic area. This combination both involves aggregation and integration. The term aggregation is here used for the combination of comparable elements across temporal and spatial scales, indicators and criteria, within a descriptor. The term integration is used for the combination of different elements (e.g., across descriptors). Both combination methods (aggregation and integration) may involve numeric calculations.

In Europe, the MSFD defines environmental status as "the overall state of the environment in marine waters, taking into account the structure, function, and processes of the constituent marine ecosystems together with natural physiographic, geographic, biological, geological and climatic factors, as well as physical, acoustic and chemical conditions, including those resulting from human activities inside or outside the area concerned."

Taking this definition into account, Borja et al. (2013) have proposed an operational definition: "GEnS is achieved when physicochemical (including contaminants, litter and noise) and hydrographical conditions are maintained at a level where the structuring components of the ecosystem are present and functioning, enabling the system to be resistant (ability to withstand stress) and resilient (ability to recover after a stressor) to harmful effects of human pressures/activities/impacts, where they maintain and provide the ecosystem services that deliver societal benefits in a sustainable way (i.e., that pressures associated with uses cumulatively do not hinder the ecosystem components in order to retain their natural diversity, productivity and dynamic ecological processes, and where recovery is rapid and sustained if a use ceases)."

This latter definition includes all MSFD descriptors. Hence, to assess whether or not GEnS has been achieved, some aggregation within and integration across the 11 descriptors is required to move from the evaluation at the level of indicators (the 56 indicators and 29 criteria described in the Commission Decision (European Commission, 2010, see also **Table 1**) to a global assessment of status, as mentioned also in Cardoso et al. (2010). The problem is how to deal with the complex task of combining a high number of indicators and descriptors. To develop a common understanding on this, it is important that Member States are transparent on (i) the process of selecting the indicators to be monitored; (ii) the approaches and combination methods they have used; and (iii) the uncertainties in their indicators and methods.

GENERAL PRINCIPLES FOR COMBINATION

Based on a literature review, we identified a number of different approaches for combining a number of variables (which could be metrics, indicators, or criteria) into an overall assessment. Some of them have been used within the WFD, others within the RSCs and some others in the MSFD. An overview of the methods is given in **Table 2**.

When considering the aggregation of indicators, an important factor to be taken into account is the reliability of the individual indicators to be aggregated. With each indicator, it is always possible to make a type I error, i.e., to get a non-GEnS result when the system in fact is in GEnS. The probability of this false positive (FP) signal varies (i) between indicators (Murtaugh, 1996), depending on the natural variability; (ii) with the amount of data used to define the indicator value; and (iii) with the target level compared to the situation in the nature. The risk of getting a FP from each of the individual indicators should affect the aggregation rule as well: if the risk of a FP is a uniform 5% per indicator, on average 1 out of 20 indicators is expected to give a FP; a problem if all indicators should in fact show GEnS. In order to come up with an aggregated assessment in which the risk level is within reasonable bounds, this aspect cannot be overlooked.

ONE-OUT, ALL-OUT (OOAO)

The OOAO approach is used in the WFD to integrate within and across Biological Quality Elements (BQEs) (CIS, 2003), in order to reach the ecological status of a water body. This approach follows the general concept that the ecological status assigned to a water body depends on the BQE with the lowest status, and consequently, the OOAO approach results in a "worst case."

A prerequisite for the aggregation of various indicators is that they are sensitive to the same pressure (Caroni et al., 2013). In such a case, different aggregation methods can be used to combine parameters (medians, means, etc.). Caroni et al. (2013) recommend an OOAO approach when the combination involves parameters/indicators that are sensitive to different pressures. The application of averaging rules may lead to biased results in those cases. The WFD Classification Guidance (CIS, 2003) also advises

Table 1 | Descriptors, criteria and indicators selected by the European Commission (2010), for ecosystem-based assessment and management of European seas, within the Marine Strategy Framework Directive.

Descriptors	Criteria	Indicators
1. **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.	1.1. Species distribution	1.1.1. Distributional range 1.1.2. Distributional pattern within the latter, where appropriate 1.1.3. Area covered by the species (for sessile/benthic species)
	1.2. Population size	1.2.1. Population abundance and/or biomass, as appropriate
	1.3. Population condition	1.3.1. Population demographic characteristics (e.g., body size or age class structure, sex ratio, fecundity rates, survival/ mortality rates) 1.3.2. Population genetic structure, where appropriate
	1.4. Habitat distribution	1.4.1. Distributional range 1.4.2. Distributional pattern
	1.5. Habitat extent	1.5.1. Habitat area 1.5.2. Habitat volume, where relevant
	1.6. Habitat condition	1.6.1. Condition of the typical species and communities 1.6.2. Relative abundance and/or biomass, as appropriate 1.6.3. Physical, hydrological and chemical conditions
	1.7. Ecosystem structure	1.7.1. Composition and relative proportions of ecosystem components (habitats and species)
2. **Non-indigenous species** introduced by human activities are at levels that do not adversely alter the ecosystems.	2.1. Abundance and state characterization of non-indigenous species, in particular invasive species	2.1.1. Trends in abundance, temporal occurrence and spatial distribution in the wild of non-indigenous species, particularly invasive non-indigenous species, notably in risk areas, in relation to the main vectors and pathways of spreading of such species
	2.2. Environmental impact of invasive non-indigenous species	2.2.1 Ratio between invasive non-indigenous species and native species in some well-studied taxonomic groups (e.g., fish, macroalgae, molluscs) that may provide a measure of change in species composition (e.g., further to the displacement of native species) 2.2.2. Impacts of non-indigenous invasive species at the level of species, habitats and ecosystem, where feasible
3. 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.	3.1. Level of pressure of the fishing activity	3.1.1. Fishing mortality (F) 3.1.2. Ratio between catch and biomass index (hereinafter "catch/biomass ratio") (if analytical assessments yielding values for F are not available)
	3.2. Reproductive capacity of the stock	3.2.1. Spawning Stock Biomass (SSB) 3.2.2. Biomass indices (if analytical assessments yielding values for SSB are not available)
	3.3. Population age and size distribution	3.3.1. Proportion of fish larger than the mean size of first sexual maturation 3.3.2. Mean maximum length across all species found in research vessel surveys 3.3.3. 95 % percentile of the fish length distribution observed in research vessel surveys 3.3.4. Size at first sexual maturation, which may reflect the extent of undesirable genetic effects of exploitation (secondary indicator)

(Continued)

Table 1 | Continued

Descriptors	Criteria	Indicators
4. 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.	4.1. Productivity of key species or trophic groups	4.1.1. Performance of key predator species using their production per unit biomass (productivity)
	4.2. Proportion of selected species at the top of food webs	4.2.1. Large fish (by weight)
	4.3. Abundance/distribution of key trophic groups/species	4.3.1. Abundance trends of functionally important selected groups/species. Detailed indicators need to be further specified, taking account of their importance to the food webs, on the basis of suitable groups/species in a region, sub-region or subdivision, including where appropriate: (i) groups with fast turnover rates (e.g., phytoplankton, zooplankton, jellyfish, bivalve molluscs, short-living pelagic fish) that will respond quickly to ecosystem change and are useful as early warning indicators, (ii) groups/species that are targeted by human activities or that are indirectly affected by them (in particular, by-catch and discards), (iii) habitat-defining groups/species, (iv) groups/species at the top of the food web, (v) long-distance anadromous and catadromous migrating species, and (vi) groups/species that are tightly linked to specific groups/species at another trophic level
5. Human-induced **eutrophication** is minimized, especially adverse effects thereof, such as losses in biodiversity, ecosystem degradation, harmful algae blooms and oxygen deficiency in bottom waters.	5.1. Nutrient levels	5.1.1. Nutrients concentration in the water column 5.1.2. Nutrient ratios (silica, nitrogen and phosphorus), where appropriate
	5.2. Direct effects of nutrient enrichment	5.2.1. Chlorophyll concentration in the water column 5.2.2. Water transparency related to increase in suspended algae, where relevant 5.2.3. Abundance of opportunistic macroalgae 5.2.4. Species shift in floristic composition such as diatom to flagellate ratio, benthic to pelagic shifts, as well as bloom events of nuisance/toxic algal blooms (e.g., cyanobacteria) caused by human activities
	5.3. Indirect effects of nutrient enrichment	5.3.1. Abundance of perennial seaweeds and seagrasses (e.g., fucoids, eelgrass and Neptune grass) adversely impacted by decrease in water transparency 5.3.2. Dissolved oxygen, i.e., changes due to increased organic matter decomposition and size of the area concerned
6. **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.	6.1. Physical damage, having regard to substrate characteristics	6.1.1. Type, abundance, biomass and areal extent of relevant biogenic substrate 6.1.2. Extent of the seabed significantly affected by human activities for the different substrate types
	6.2. Condition of benthic community	6.2.1. Presence of particularly sensitive and/or tolerant species 6.2.2. Multi-metric indexes assessing benthic community condition and functionality, such as species diversity and richness, proportion of opportunistic to sensitive species 6.2.3. Proportion of biomass or number of individuals in the macrobenthos above some specified length/size 6.2.4. Parameters describing the characteristics (shape, slope and intercept) of the size spectrum of the benthic community

(Continued)

Table 1 | Continued

Descriptors	Criteria	Indicators
7. **Permanent alteration of hydrographical conditions** does not adversely affect marine ecosystems.	7.1. Spatial characterization of permanent alterations	7.1.1. Extent of area affected by permanent alterations
	7.2. Impact of permanent hydrographical changes	7.2.1. Spatial extent of habitats affected by the permanent alteration 7.2.2. Changes in habitats, in particular the functions provided (e.g., spawning, breeding and feeding areas and migration routes of fish, birds and mammals), due to altered hydrographical conditions
8. Concentrations of **contaminants** are at levels not giving rise to pollution effects.	8.1. Concentration of contaminants	8.1.1. Concentration of the contaminants mentioned above, measured in the relevant matrix (such as biota, sediment and water) in a way that ensures comparability with the assessments under Directive 2000/60/EC
	8.2. Effects of contaminants	8.2.1. Levels of pollution effects on the ecosystem components concerned, having regard to the selected biological processes and taxonomic groups where a cause/effect relationship has been established and needs to be monitored 8.2.2. Occurrence, origin (where possible), extent of significant acute pollution events (e.g., slicks from oil and oil products) and their impact on biota physically affected by this pollution
9. **Contaminants in fish and other seafood for human consumption** do not exceed levels established by Community legislation or other relevant standards.	9.1. Levels, number and frequency of contaminants	9.1.1. Actual levels of contaminants that have been detected and number of contaminants which have exceeded maximum regulatory levels 9.1.2. Frequency of regulatory levels being exceeded
10. Properties and quantities of **marine litter** do not cause harm to the coastal and marine environment.	10.1. Characteristics of litter in the marine and coastal environments	10.1.1. Trends in the amount of litter washed ashore and/or deposited on coastlines, including analysis of its composition, spatial distribution and, where possible, source 10.1.2. Trends in the amount of litter in the water column (including floating at the surface) and deposited on the sea- floor, including analysis of its composition, spatial distribution and, where possible, source 10.1.3. Trends in the amount, distribution and, where possible, composition of micro-particles (in particular micro- plastics)
	10.2. Impacts of litter on marine life	10.2.1. Trends in the amount and composition of litter ingested by marine animals (e.g., stomach analysis)
11. Introduction of **energy, including underwater noise**, is at levels that do not adversely affect the marine environment.	11.1. Distribution in time and place of loud, low and mid frequency impulsive sounds	11.1.1. Proportion of days and their distribution within a calendar year over areas of a determined surface, as well as their spatial distribution, in which anthropogenic sound sources exceed levels that are likely to entail significant impact on marine animals measured as Sound Exposure Level (in dB re 1 μPa 2.s) or as peak sound pressure level (in dB re 1 μPa_{peak}) at 1 m, measured over the frequency band 10 Hz to 10 kHz
	11.2. Continuous low frequency sound	11.2.1 Trends in the ambient noise level within the 1/3 octave bands 63 and 125 Hz (center frequency) (re 1 μPa RMS; average noise level in these octave bands over a year) measured by observation stations and/or with the use of models if appropriate

Table 2 | Approaches for combining different metrics, indicators or criteria to assess the status, including the advantages and disadvantages of each approach, as considered by the authors.

General approach	Details of method	Advantages	Disadvantages
One-out all-out (OOAO) principle (CIS, 2003; Borja et al., 2009a; Borja and Rodríguez, 2010; Ojaveer and Eero, 2011; Caroni et al., 2013)	All variables have to achieve good status	Most comprehensive approach. Follows the precautionary principle	Trends in quality are hard to measure. Does not consider weighting of different indicators and descriptors. Chance of failing to achieve good status very high
	As a variation, Tueros et al. (2009) proposed the Two-out all-out: if two variables do not meet the required standard, good status is not achieved	More robust compared to OOAO approach	See above
Averaging approach (Ojaveer and Eero, 2011; Shin et al., 2012)	*Non-weighted*: Variable values are combined, using the arithmetic average or median	Indicator values can be calculated at each level of aggregation. Recommended when combined parameters are sensitive to a single pressure	Assumes all variables are of equal importance
	Weighted: Like the previous method, with different weights assigned to the various variables *Hierarchical*: With variables defined at different hierarchical levels	Reflects the links between descriptors and avoids double counting Reflects the hierarchy among descriptors and avoids double counting Different calculation rules can be applied at different levels	High data requirements. Problem of agreeing on weights Problem of agreeing on hierarchy
Conditional rules (Tueros et al., 2009; Simboura et al., 2012; Breen et al., 2012)	A specific proportion of the variables have to achieve good status	Focuses on the key aspects (i.e., biodiversity descriptors)	Assumes that GEnS is well represented by a selection of variables
Scoring or rating (Borja et al., 2004, 2010, 2011b; Birk et al., 2012)	Sum of weighted scores	Different weights can be assigned to the various elements	Problem of agreeing on weights. Metrics may not be sensitive to the same pressures
Multimetric approaches (Rice et al., 2010; Borja et al., 2011a; Birk et al., 2012)	Multi-metric indices	Integrates multiple indicators into one value. May result in more robust indicators, compared to indicators based on single parameters	Correlations between parameters can be an issue. Results are hard to communicate to managers. Metrics may not be sensitive to the same pressures
Multi-dimensional approaches (Shin et al., 2012)	Multivariate analyses	No need to set rigid target values, since values are represented within a domain	Results are hard to communicate to managers
Decision tree (Borja et al., 2004, 2009b, 2013)	Integrating elements into a quality assessment using specific decision rules	Possible to combine different types of elements, flexible approach	Only quantitative up to a certain level
Probabilistic (Barton et al., 2008, 2012; Lehikoinen et al., 2013, 2014)	Bayesian statistics	Produces a probability estimate of how likely the area is in GEnS; managers can decide the acceptable undertainty	Difficult to calculate
High-level integration (HELCOM, 2010; Borja et al., 2010, 2011b; Halpern et al., 2012; Tett et al., 2013)	Assessment results for three groups: biological indicators, hazardous substances indicat ors and supporting indicators, each applying OOAO	Reduces the risks associated with OOAO while still giving an overall assessment	Technical details

GEnS, Good environmental status.

to use OOAO when combining parameters/indicators that are sensitive to different pressures.

Borja et al. (2009a) discussed the challenge of assessing ecological integrity in marine waters, and suggest that simple approaches, such as the "OOAO" principle of the WFD, may be a useful starting point, but eventually should be avoided. The ecological integrity of an aquatic system should be evaluated using all information available, including as many biological ecosystem elements as is reasonable, and using an ecosystem-based assessment approach. The OOAO rule can be considered a rigorous approach to the precautionary rule, in an ideal world where the status based on each BQE can be measured without error. It results in very conservative assessments (Ojaveer and Eero, 2011). In practice, the inevitable uncertainty associated with monitoring and assessment for each metric and BQE leads to problems of probable underestimation of the true overall status. The OOAO principle has therefore been criticized as it increases the probability of committing a false positive error, leading to an erroneous downgrading of the status of a water body as it has been observed especially within the WFD (Borja and Rodríguez, 2010; Ojaveer and Eero, 2011; Borja et al., 2013; Caroni et al., 2013). In the case of the MSFD, with such large number of descriptors, criteria and indicators, the probability of not achieving good status becomes very high and, probably, unmanageable in practical terms (Borja et al., 2013).

Alternative methods for integrating multiple BQEs in the WFD are currently being considered (Caroni et al., 2013).

AVERAGING APPROACH

The averaging approach is the most commonly used method to aggregate indicators (Shin et al., 2012) and consists of simple calculations, using methods such as arithmetic average, hierarchical average, weighted average, median, sum, product or combinations of those rules, to come up with an overall assessment value.

Ojaveer and Eero (2011) showed that in cases where a large number of indicators is available, the choice of e.g., either medians or averages in aggregating indicators did not substantially influence the assessment results. However, this might not necessarily be the case when only a few indicators are available. In such a situation, the result will depend to a larger degree on the distribution of the values involved. A skewed distribution reflecting some major factors and a few ones with very different values will result in very different assessment results for the median compared to assessments based on means. Apart from the mathematical applicability of either method based on the underlying data (e.g., homoscedasticity), the choice of the actual averaging method may be driven by policy decisions focusing on either central trends without much attention to extreme values (median) or focusing on weighting the individual values by their magnitude (arithmetic mean).

The way the indicators are hierarchically arranged influences the assessment results as well, but Ojaveer and Eero (2011) found that these effects were considerably less important than the effects of applying different aggregation rules.

Differential weighting applied to the various indicators can be used when calculating means or medians. An adequate basis for assigning weights is not always available and in such cases an equal weight is recommended by Ojaveer and Eero (2011). Assigning weights often involves expert judgment, and Aubry and Elliott (2006) point out that in some cases, expert opinions on weights can show important divergence.

CONDITIONAL RULES

Conditional rules (a specific proportion of the variables have to achieve good status) are an approach where indicators can be combined in different ways for an overall assessment, depending on certain criteria. This provides an opportunity to use expert judgment when combining indicators, in a transparent way. An example of this approach is the application of a conditional rule of at least two out of three indicators (one biotic index and two structural or diversity indices) should pass the threshold in order to achieve GEnS for benthic community condition under D6 in Hellenic waters (Simboura et al., 2012). Tueros et al. (2009) present another example of the conditional rule in which when integrating water and sediment variables into an overall assessment of the chemical status and only one sediment or water variable does not meet the objective, while the rest of the variables meet, the final chemical status achieves the objective. This work was also mentioned under the "two out, all out" approach considering the case when two variables do not meet the objective and the final status fails.

Breen et al. (2012) used several risk criteria rules and worst-case or integrated approaches when combining evidence before a final assessment. Following Cardoso et al. (2010) the integrated approach was applied to Biodiversity, Non-indigenous species, Eutrophication and Seafloor Integrity descriptors, while all other descriptors used a worst case approach following the OOAO principle whereby if one set of evidence suggested that the risk was "high" then "high" was automatically assessed for the entire descriptor.

SCORING OR RATING

In this method different scores are assigned to a status level (for example, ranging from 1 to 5), for a number of different elements. The scores are summed up to derive a total score which is then rated according to the number of elements taken into account. Different weights can be assigned to the various elements. This method was proposed by Borja et al. (2004) to calculate an integrative index of quality and is the basis of many multimetric indices used within the WFD and the MSFD combining different parameters or metrics using the weighted scoring or rating rule into one integrative multimetric index (Birk et al., 2012). It must be recognized here that this approach implies the score values being on a cardinal scale and acting as weighting factors. Otherwise, using an ordinal scale for the scores, summing up the individual elements is mathematically not defined.

Another example is the method developed by Borja et al. (2010, 2011b) for a cross-descriptor integration, combining the 11 descriptors of MSFD based on the WFD, HELCOM (2009a,b, 2010) and OSPAR (2010, 2012) experiences. An Ecological Quality Ratio (EQR) was calculated for each indicator of the various MSFD descriptors, with the EQR for the whole descriptor being the average value of the EQR of the indicators. Then, by multiplying the EQR with the percent weight assigned to each descriptor (and summing up to 100), an overall environmental status value was derived.

MULTIMETRIC INDICES TO COMBINE INDICATORS

Within the WFD there are many examples of multimetric indices developed for different biological elements, driven by the need to fulfill the detailed requirements of the WFD (see Birk et al., 2012 for a complete synthesis).

In addition, within the MSFD, the European Commission established a number of Task Groups consisting of technical experts to help inform the discussions on how to reach a common understanding of the 11 descriptors. Hence, Task Group 6 report on seafloor integrity (Rice et al., 2010) recommends the use of multimetric indices or multivariate techniques for integrating indicators of species composition attributes of this descriptor, such as diversity, distinctness, complementarity/(dis)similarity, or species-area relationships.

There are various other examples of multi-metric indices used to assess the status of the macrobenthos (see Borja et al., 2011a for an overview). Multimetric methods to combine multiple parameters in one assessment may result in more robust indicators, compared to indicators based on single parameters. However, scaling of a multimetric index may be less straightforward, and ideally the various parameters should not be inter-correlated (e.g., the discussion on the TRIX index in Primpas and Karydis, 2011).

MULTIDIMENSIONAL APPROACHES

Multivariate methods, such as Discriminant Analysis or Factor Analysis combine parameters in a multi-dimensional space. For assessment purposes, areas need to be classified into groups of GEnS and non-GEnS.

Multivariate methods have the advantage of being more robust and less sensitive to correlation between indicators. However, interpretation is less intuitive than other methods, as information on individual indicators in each ecosystem is lost (Shin et al., 2012) and links to management options are less obvious.

DECISION TREE

Decision trees provide the opportunity to apply different, specific, rules to combine individual assessments into an overall assessment. A decision tree allows implementing individual rules at each of its nodes and thus incorporates arbitrary decisions at each step within the decision tree. The decision rules can be quantitative or qualitative as well as based on expert judgment. This gives room for a high degree of flexibility in reaching the final assessment and can thus be used where the other principles fail to represent the intricate interactions, feedback loops and dependencies involved in ecosystem functioning between the ecosystem components.

A simple version of a decision tree involves only having a few conditional rules where a specific proportion or certain individually specified indicators have to achieve good status in order to achieve GEnS. Borja et al. (2013) implicitly propose using this kind of decision tree when they take the view that for biodiversity (D1) to be in good status, all other descriptors must be in good status and if one of the pressure descriptors fails, then D1 also fails.

Borja et al. (2004, 2009b) describe a methodology that integrates several biological elements (phytoplankton, benthos, algae, phanerogams, and fishes), together with physicochemical elements (including pollutants) into a quality assessment. The proposed methodologies accommodate both WFD and the MSFD. They suggest that the decision tree should give more weight to individual elements taking into account the spatial and temporal variability and the availability of accurate methodologies for some of them (i.e., benthos) and to individual assessment methods which have been used broadly by authors other than the proposers of the method, tested for several different human pressures, and/or intercalibrated with other methods.

PROBABILISTIC APPROACH

Each of the indicator results are uncertain, due to several factors e.g., natural variation in the sampling sites, random variation in the samples, insufficient scientific understanding about what should be the reference value for good status, etc. Some indicators are bound to include more uncertainty that others, due to differences in the amount of data used, the extent of scientific understanding regarding the issue, and the amplitude of natural variation. If these uncertainties can be approximated, this gives rise to the possibility of taking this information into account when integrating the indicators. The more uncertain indicators will get less weight in the integrated assessment, while the more certain ones will be more reliable and hence get more weight. The calculus of the integrated assessment can be based on Bayesian statistics, giving transparent and coherent rules by which the final score is calculated.

This approach can be combined to one or several of the above-mentioned approaches: for example, conditional rules can be set in addition to the probabilistic integration rule to include expert judgment; and the principles outlined in the decision tree approach can be applied as well.

Barton et al. (2012) demonstrate how to use the probabilistic approach in the DPSIR framework in the case of eutrophication management. There are several other examples in the recent literature about how to evaluate various management measures under uncertainty to optimize one target, such as eutrophication (Barton et al., 2008; Lehikoinen et al., 2014) and oil spill severity (Lehikoinen et al., 2013). This approach could be expanded to include several descriptors or indicators.

Probabilistic combination of uncertain indicators would naturally lead to a probability estimate of how likely it is that a marine area is in GEnS; we would, for example, end up with an estimate that the sea area is in GEnS with 70% probability. The managers would then have to decide how much uncertainty they are willing to tolerate; i.e., are they happy if the probability of GEnS is above 50%, or whether they want a higher certainty?

HIGH-LEVEL INTEGRATION

An example of a high-level integration, where assessments for several ecosystem components are merged into a final assessment, is the HELCOM-HOLAS project (HELCOM, 2010). The report presents an indicator-based assessment tool termed HOLAS

("Holistic Assessment of Ecosystem Health Status"). The indicators used in the thematic assessments for eutrophication (HEAT), hazardous substances (CHASE) and biodiversity (BEAT) were integrated into a Holistic Assessment of "ecosystem health." The HOLAS tool presented assessment results for three groups: biological indicators, hazardous substances indicators and supporting indicators, and then applied the OOAO principle on the assessment results of those three groups for the final assessment (**Figure 1**).

This approach, which includes the selection of an agreed reduced set of indicators and agreed weighting rules, could be considered a pragmatic compromise, reducing the risks associated with OOAO while still giving an overall assessment.

An example of such a high level aggregation is the integrative method of Borja et al. (2010, 2011b), which includes a weighted scoring or rating method proposed for the MSFD in the southern Bay of Biscay. After aggregating the indicators within each descriptor, each descriptor was weighted according to the human pressure supported by the area. Then the value of each descriptor (i.e., an EQR) was multiplied by the weighting and added to obtain a final value between 0 and 1, being 0 the worst environmental status and 1 the best. This high-level integration was done at spatial and temporal scale. Although these authors combine values across descriptors, leading to a single value of environmental status, it could also be reported as "x out of 11 descriptors" having reached GEnS. In both cases, this allows to take management measures on those human activities impacting more in some of the descriptors or indicators not achieving good status, as shown in Borja et al. (2011b).

Halpern et al. (2012) developed another method, based more upon human activities and pressures, which presents a high-level integration at country level, using internationally available

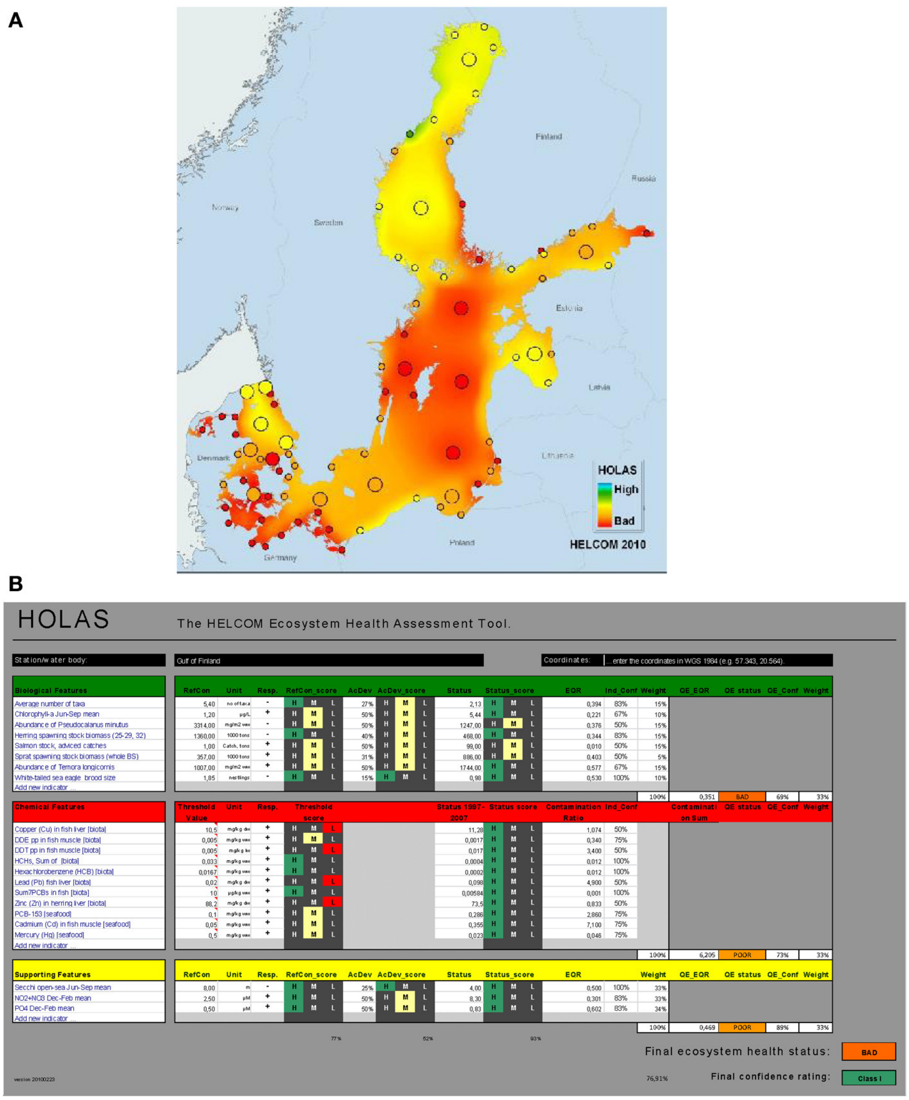

FIGURE 1 | (A) Example of an integrated assessment of ecosystem health in the Baltic Sea 2003–2007 based on the HOLAS tool. **(B)** Screenshot to illustrate how the HOLAS classification tool for the Gulf of Finland works. See HELCOM (2010) for details. Courtesy by Helsinki Commission.

datasets (Ocean Health Index http://www.oceanhealthindex.org). Similarly, Micheli et al. (2013) looked at cumulative impacts to the marine ecosystems of the Mediterranean and the Black Sea as a whole, while producing impact scores and maps for seven ecoregions and the territorial waters of EU Member states.

A Baltic Sea Health Index (BSHI) will be developed based on: (i) the existing HELCOM toolbox (HEAT, BEAT, CHASE and HOLAS), the MSFD (European Commission, 2008, 2010), and (ii) the Ocean Health Index (Halpern et al., 2012).

Finally, there is a recent high-level integration example in Tett et al. (2013), for the North Sea, which includes five steps in the calculation: (i) identify (spatial extent) of ecosystem; (ii) identify spatial granularity and extent of repetitive temporal variability, and decide how to average or integrate over these; (iii) select state variables; (iv) plot trajectory in state space and calculate Euclidian (scalar) distance from (arbitrary) reference condition; and (v) calculate medium-term variability about trend in state space, and use this variability as proxy for (inverse) resilience.

CONSIDERATIONS AND RECOMMENDATIONS WHEN USING SPECIFIC RULES

As shown in the previous section, the considerations to be used in combining values and assessing the environmental status are not easily defined. From the lessons learned above, some guidance can be offered:

(1) OOAO is appropriate when:

- Legal criteria are involved, (e.g., contaminants exceeding legal quality standards, species or habitats failing favorable conservation status under Birds or Habitat Directives, commercial fish stocks failing Maximum Sustainable Yield targets under Common Fisheries Policy).
- Different pressures are addressed (but in that case other methods can be also used).
- There is an impact or risk on a future impact.
- The precautionary principle is applied (e.g., in the case when little information from only a few indicators is available).

(2) OOAO cannot be used:

- In cases where indicators show a high level of uncertainty, when various indicators are sensitive to the same pressure, etc. In practice, the uncertainty associated with monitoring and assessment for each indicator/descriptor leads to problems of probable underestimation of the true overall class. Hence, if the error associated to the method used to assess the status of each indicator/descriptor is too high the OOAO approach is not advisable.
- Note: Often, not all indicators are in the same state of development, or are scientifically sound and fully tested. In some cases P-S-I (Pressure-State-Impact) relations are uncertain. Also, sometimes multiple indicators are used to describe state. While not all of those indicators may be equally important or even comparable, this is done to include indicators that are used as supportive indicators, where P-S-I relations are uncertain. In those cases an aggregation rule such as OOAO should not be applied.

(3) A "two out, all out" approach can be considered in cases where several methods are combined in one assessment; e.g., when several matrices are used in pollutants to give a broader view of the status (e.g., pollutants in water for an instant picture, pollutants in sediments or biota for a time-integrated result, Tueros et al., 2009).

(4) Averaging is appropriate when combined variables or indicators are of equal importance or sensitive to the same pressure.

(5) Scoring or decision tree approaches are appropriate when:

- The methods to assess the status of the different indicators/descriptors are in different levels of development. In this case, consider giving more weight to those indicator/assessment methods which have been: (i) used broadly by authors other than the proposers of the method; (ii) tested for several different human pressures; and/or (iii) intercalibrated with other methods.
- It is important to be able to track the different steps involved in the assessment, making the path to the final assessment result transparent.
- Note: Consider different weights for individual indicators/descriptors taking into account the relationship with the pressures within the assessment (sub)region. E.g., if the area is under high fishing pressure the most affected descriptors will be D1, D3, D4, D6 and D11; in turn, D2, D5, D7, D8, D9 and D10 will be less affected.

(6) Probabilistic approach:

- Consider carefully the uncertainties related to all of the various parts of the problem; be sure not to overestimate the well-known uncertainties (e.g., natural variance and sampling bias) and underestimate the poorly known uncertainties (e.g., insufficient knowledge or competing hypotheses about ecological interactions; combined effects of various pressures that may be strengthen or weaken each other, etc.).
- Consider using expert knowledge in evaluating the various uncertainties.
- If using expert judgment to weigh the different indicators in addition to the uncertainty estimate, make sure that the weighing is based on the relative importance of the indicators, not on the perceived uncertainty; otherwise you will end up double counting the effect of uncertainty in the final evaluation.

(7) Multimetric and multivariate methods are appropriate when:

- Integrating several indicators of species composition or several indicators of eutrophication or seafloor integrity (e.g., in D1, D5, D6).
- It is advisable to verify that stakeholders and managers can understand the interpretation of the results, and results must be presented in a clear way.

(8) For any of the described methods take into account that:

- Using as many ecosystem components/indicators/criteria as reasonable and available will make the analysis more robust.
- Integrate across state descriptors (D1, D3, D4, D6) differently than across pressure descriptors (D2, D5, D7, D8, D9, D10, D11), giving higher weight to state-based descriptors.

APPLICATION OF COMBINATION RULES IN ASSESSMENTS

As shown above, the WFD focuses on the structure of the ecosystem using a limited number of biodiversity components (the BQEs), that are combined through the precautionary OOAO approach (Borja et al., 2010). In contrast, the MSFD can be considered to follow a "holistic functional approach," as it takes into account not only structure (biodiversity components, habitats), but also function (e.g., food webs, seafloor integrity) and processes (e.g., biogeochemical cycles) of the marine ecosystems. The MSFD also uses descriptors that not only relate to biological and physicochemical state indicators but also to pressure indicators (Borja et al., 2010, 2013). The MSFD requires the determination of GEnS on the basis of the qualitative descriptors in Annex I, but does not specifically require one single GEnS assessment, in contrast to the WFD.

There are many methodological challenges and uncertainties involved in establishing a holistic ecosystem assessment, when it is based on the large number of descriptors, associated criteria and indicators defined under the MSFD. The choice of indicator aggregation rules is essential, as the final outcome of the assessment may be very sensitive to those indicator aggregation rules (Ojaveer and Eero, 2011; Borja et al., 2013; Caroni et al., 2013). As shown in the previous section, different methodologies can be applied for aggregating indicators, which vary, amongst others, in the way the outliers influence the aggregate value.

When aggregating indicators most researchers agree that multiple accounting should be avoided. For example, phytoplankton indicators under D1 should be indicative of biodiversity state while under D5 it should be an estimator of the level of eutrophication. Similarly, macroinvertebrates under D1 should represent biodiversity state and under D6 also the state change from pressures on the seafloor. In these cases, although the datasets used could be the same, the main characteristics of the indicators to be used within each descriptor should be different, e.g., the value of macroinvertebrates indicators under D1 (rarity of species, endangered species, engineer species presence, etc.) and the condition of benthic community under D6 (ratio of opportunistic/sensitive, multimetric methods to assess the status, etc.). Of course, for aggregating indicators within the same criterion it is important that all indicators have the same level of maturity and that sufficient data are available.

There are at least four levels of combination required to move from evaluation of the individual metrics or indicators identified by the Task Groups to an assessment of GEnS (Cardoso et al., 2010). As an example, using D6 (Seafloor integrity), **Figure 2** shows: (i) aggregation of metrics/indices within indicators (see names of indicators in **Table 1**); (ii) aggregation of indicators within the criteria of a descriptor (for complex descriptors), e.g., criteria 6.1 (physical damage) and 6.2 (condition of benthic community); (iii) status across all the criteria of a descriptor; and (iv) integration of status across all descriptors.

As one moves up the scale from metric/indicator level to overall GEnS, the diversity of features that have to be combined increases rapidly (**Figure 2**). This poses several challenges arising from the diversity of metrics, scales, performance features (sensitivity, specificity, etc.) and inherent nature (state indicators, pressure indicators, impact indicators) of the metrics that must be integrated.

AGGREGATION OF INDICATORS AND CRITERIA (COMBINATION WITHIN A DESCRIPTOR)

Cardoso et al. (2010) summarize the methods for an integration within a MSFD descriptor, categorizing them into two wider

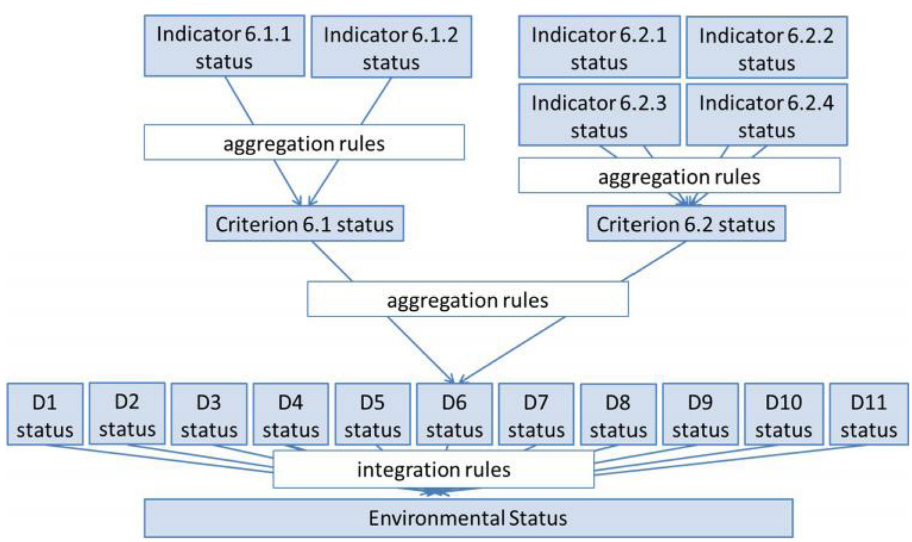

FIGURE 2 | Diagram of a possible approach for aggregation of indicators and criteria and integration of descriptors (D), using D6 as an example. For indicators and criteria description, see **Table 1**.

categories: (i) integrative assessments combining indicators and/or attributes appropriate to local conditions; and (ii) assessment by worst case (in this context, "worst case" means that GEnS will be set at the environmental status of the indicator and/or attribute assessed at the worst state for the area of concern).

Table 3 summarizes the approaches to aggregate attributes within each descriptor. In some cases the MSFD Task Groups propose deconstructing the ecosystem into "descriptor indicators" and then recombining them again to give a pass/fail for the GEnS, using (in four cases) the OOAO principle (**Table 3**). Borja et al. (2013) emphasize that such a "deconstructive structural approach" makes large assumptions about the functioning of the system and does not consider the weighting of the different indicators and descriptors. It implies that recombining a set of structural attributes gives an accurate representation of the ecosystem functioning.

An example of this accurate representation is shown by Tett et al. (2013), who assess the ecosystem health of the North Sea, using different attributes and components of the ecosystem. These components include structure or organization, vigor, resilience, hierarchy and trajectory in state space. All the information from the different components are combined and synthesized for a holistic approach to assess the ecosystem health.

Other approaches have been used in aggregating indicators within each descriptor. For example, Borja et al. (2011b) use the biodiversity valuation approach, in assessing biodiversity within the MSFD, integrating several biodiversity components (zooplankton, macroalgae, macroinvertebrates, fishes, cetaceans and seabirds). Biodiversity valuation maps aim at the compilation of all available biological and ecological information for a selected study area and allocate an integrated intrinsic biological value to the subzones (Derous et al., 2007). Details on valuation methodology can be consulted in Pascual et al. (2011) (see Figure 4 in that paper). This methodology provides information for each of the components and their integrative valuation, together with the reliability of the result, taking into account spatial and temporal data availability (Derous et al., 2007). The advantage of this method is that the current information used to valuate biodiversity can be adapted to the requirements of the MSFD indicators. Moreover, this method can avoid duplication of indicators in two descriptors (e.g., D1 and D6), since the metrics used could be different. This information can be converted into environmental status values, as shown in Borja et al. (2011b).

Table 3 | Summary of Task Group approaches to aggregate attributes within a Descriptor (Cardoso et al., 2010).

Aggregation of attributes	Descriptor
Integrative assessments (combining attributes appropriate to local conditions)	D1 Biodiversity
	D2 Non-indigenous species
	D5 Eutrofication
	D6 Seafloor integrity
Assessment by worst case (Descriptor not in good status if any attribute is not OK)	D3 Commercial fish (3 attributes)
	D4 Food webs (2 attributes)
	D8 Contaminants (3 attributes)
	D9 Contaminants in fish (1 attribute)
	D10 Litter (3 attributes)
	D11 Energy and noise (3 attributes)

INTEGRATION OF DESCRIPTORS (COMBINATION ACROSS DESCRIPTORS)

Discussion on how to integrate the results of each descriptor into an overall assessment of GEnS for regions or subregions was not part of the Terms of Reference for the Task Groups. However, work within Task Group 6 (Sea floor integrity) identified a method for integration and assessment that might also be appropriate, if applied across all descriptors, at a regional scale (Cardoso et al., 2010). As these authors pointed out, cross-descriptor integration at the scale of (sub)regional seas runs the risk of blending and obscuring the information that is necessary to follow progress toward GEnS and to inform decision-makers about the effects and the efficiency of policies and management. It may lead to masking of problems within specific descriptors.

Borja et al. (2013) describe at least 8 options to determine GEnS in a regional sea context (**Table 4**). These authors detail the concept behind these options, and propose the decision rule more adequate for the assessment method to be used, depending on the circumstances i.e., data availability, lack of monitoring, etc. In addition, these authors consider what type and amount of data are required, and then discuss the pros and cons of the different options. The implementation of a complex directive, such as the MSFD, requires a high amount of data to assess the environmental status in a robust way. Hence, the options from 1 to 8 proposed in **Table 4** are sequentially less demanding of new data, and the degree of detailed environmental assessment is also decreasing.

As such, Option 1, which is most similar to the WFD approach, deconstructs GEnS into the 11 descriptors and then into the component indicators, assessing each components for each area before attempting to produce an overall assessment (**Table 4**). However, having a complete dataset covering all descriptors and indicators for the assessment is difficult, if not impossible to achieve in practical terms. The use of pressure maps as an estimator of the environmental status and possible impacts to marine ecosystems could be considered instead (see **Table 4**). This would, however, build on the substantial assumption that the level of pressure is adequately representing the current state on all different levels of ecosystem components. Option 7, in contrast, only uses published data for the activities, and then infers a static relationship between activity, pressures, state changes and impacts both on the natural and the human system. Here, the number of underlying assumptions is even larger than using pressure maps, since the method relies on predefined and static DPSIR relations. Between these extremes, there are several intermediate options to integrate and present information, each with its own requirements, pros and cons (**Table 4**).

Table 4 | Options for determining if an area/regional sea is in Good Environmental Status (GEnS) (modified from Borja et al., 2013).

Option	Decision rule	Data requirements	Pros	Cons	Examples in place
Either: 1. Fulfilling all the indicators in all the descriptors	All indicators are met irrespective of weighting (OOAO)	Data needed for all aspects on regional seas scale	Most comprehensive approach	Unreasonable data requirements; all areas will fail on at least one indicator; may include double-counting	None
Or: 2. Fulfilling the indicators in all descriptors but as a weighted list according to the hierarchy of the descriptors	Agreeing the weighting	Data needed for all aspects on regional seas scale	Reflects the interlinked nature of the descriptors and avoids double counting	Unreasonable data requirements; problem of agreeing the weighting	Aubry and Elliott, 2006; HELCOM, 2010; Borja et al., 2011b
Or: 3. Fulfilling the indicators just for the biodiversity descriptor and making sure these encompass all other quality changes	All biodiversity indicators are met irrespective of weighting	Data needed for all components of biodiversity	Focuses on the main aspect	Assumes that the biodiversity descriptor really does encompass all others	Feary et al., 2014
Or: 4. Create a synthesis indicator which takes the view that "GEnS is the ability of an area to support ecosystem services, produce societal benefits and still maintain and protect the conservation features"	Integration of the information from different descriptors and indicators, and evaluation of the overall benefits	Data needed for the indicators included in that synthesis indicator, valuation of the ecosystem services and benefits	Fulfills the main aim of marine management (see text)	Requires a new indicator and an agreement in the way of integrate the information; trade-offs between ecosystem services and their beneficiaries require either economic, ethical or political evaluation and decision, and cannot be based only on ecological knowledge	Borja et al., 2011b
Or: 5. Have a check-list (ticking boxes) of all the aspects needed	Then if an area has e.g., more than 60% of the boxes ticked then it is in GEnS	An expert judgment approach, based on "probability of evidence"	It may reflect the state of the science; if done rigorously then it may be the easiest to implement	It may be too subjective (i.e., based on soft intelligence)	Bricker et al., 2003; Ferreira et al., 2011
Or: 6. Have a summary diagram such as a spiders-web diagram showing the 'shape of GEnS according to several headline indicators'	The shape of the diagram		Easy to understand and show to managers	The decision on when GEnS is achieved	Halpern et al., 2012
Or: 7. Not reporting the environmental status but only the list of pressures (i.e., on the premise that if an area has no obvious pressures then any changes in the area must be due to natural changes which are outside the control of management)	No pressures in an area sufficient to cause adverse effects	Quantitative maps of pressures	Can be derived by national databases, mapping, pressure lists	Relates to "cause" rather than "effect," difficult to set boundaries between pressure status classes: is it sufficient to base the assessment on the list of pressures, while those can have very different spatial extent and strength?	Aubry and Elliott, 2006; Halpern et al., 2008; Korpinen et al., 2012; Solheim et al., 2012
Or: 8. A combination of all/some of these when there are insufficient data in some areas or for some descriptors or indicators		Combination of pressures and descriptors data	Information available from Member States reports	Either requires too much information (hence unreasonable) or too little (hence inaccurate)	None

OOAO, "one out, all out" principle.

One-out, all-out (OOAO)

Although the MSFD describes the GEnS individually for each of the 11 descriptors, this does not necessarily imply the ability to have GEnS at the level of all the descriptors, nor does it mean that each descriptor should necessarily be graded individually in a binary way (i.e., good or not good environmental status) (Borja et al., 2013).

It could be argued that the 11 descriptors together summarize the way in which the ecosystem functions in terms of the MSFD view. As Member States have to consider each of the descriptors to determine good environmental status, this could be interpreted as a requirement to achieve GEnS for each of these descriptors. In that case, applying OOAO is the only integration method that can be applied to arrive at an overall assessment of GEnS, leading to a high probability of not achieving GEnS.

This assumes that the 11 descriptors, and the associated indicators, can be considered a coherent and consistent framework that adequately reflects the environmental status. In that situation, state descriptors not achieving GEnS would be accompanied by pressure descriptors not achieving GEnS, if the reaction of the ecosystem components is immediate, acting on the same time scale as the pressures. If this is not the case, for example if a pressure descriptor (e.g., D5 or D8) indicates that the level of the pressure is too high to achieve GEnS, while state descriptors (e.g., D1 or D4) do not reflect this, there is clearly an inconsistency in the assumed MSFD assessment framework, indicating that it does not capture delayed responses of state indicators to changing pressure indicators. That could be interpreted as a need for further research on the nature of P-S-I relations and the consistency in environmental targets for the descriptors involved, since our current state of knowledge on quantitative causal relations between pressures, state changes and impacts is limited. In addition, nearly all ecosystem components are subject to the true cumulative effects of many simultaneous pressures related to a range of human activities (Crain et al., 2008; Stelzenmüller et al., 2010; Knights et al., 2013). This means that, for some descriptors at least, there is a large scientific uncertainty associated with the definition of environmental targets and GEnS. Uncertainties in target setting, in the performance of an action (e.g., ecosystem state post-management) or in the contribution of individual driver(s) causing state change can undermine decision making when implementing environmental policy and can limit our ability to identify what should be managed, and what the impact of management might be (Knights et al., 2014). Consequently, developing a consistent assessment framework for all descriptors and indicators is an extremely challenging task, and using the OOAO approach is not appropriate.

Alternative approaches

The usefulness of integrating descriptors to one single value (overall GEnS assessment based on combination of the 11 descriptors) is under discussion by the Member States and the European Commission groups for the implementation of the MSFD. An argument against integration across descriptors is that it may not be informative any more since it results in loss of information at a crucial level where different elements are combined that cannot be integrated without major concessions.

The abovementioned groups have suggested that an integration across the biodiversity-related descriptors (D1, D2, D4, D6) might be an option, splitting those descriptors into various groups (e.g., functional or species groups). If a species or species group is assessed under more than one descriptor different aspects should be considered (e.g., chlorophyll a under D5 and phytoplankton species composition under D1).

However, if an integration across all descriptors is decided, Borja et al. (2010) suggest that the 11 descriptors are hierarchical and do not have an equal weighting when assessing the overall GEnS. Hence, Borja et al. (2013) suggest that for biodiversity (D1) to be fulfilled requires all others to be met and similarly if one of the stressor or pressure-related descriptors (e.g., D11, energy including noise) fails then by definition the biodiversity will be adversely affected at some point. This approach addresses the conceptual drawback of the OOAO principle and allows to have delayed responses to changing pressure regimes without drawing false conclusions and still being precautionary.

In addition to the problem of combining indicators (seen in the previous section) and descriptors the MSFD requires Member States to integrate and geographically scale-up the assessments at the level of a region or subregion (Borja et al., 2010). This differs strongly from the approach under the WFD, which is restricted to quality assessments at the scale of a water body (Hering et al., 2010). This means that the GEnS assessments of the different Member States within a regional sea need to be comparable and should avoid anomalies at the borders of Member States in order to enable synthesizing of the assessments into a region-wide assessment (Borja et al., 2013). This requires both comparable methods and associated combination rules to ensure minimum standards for GEnS reporting across Member States. As such, we advocate a set of common principles (expanded from Claussen et al., 2011, as shown in Borja et al., 2013):

- The combination across levels of different complexity should accommodate different alternatives, i.e., aggregation below descriptor level (across indicators within criteria, and criteria within descriptors, as shown in the previous section) and can certainly differ from descriptor level integration.
- Integration across state descriptors (D1, D3, D4, D6) should be done differently than across pressure descriptors (D2, D5, D7, D8, D9, D10, D11), but avoiding double counting of indicators in different descriptors (e.g., phytoplankton under D1 and D5, macroinvertebrates under D1 and D6).
- Consideration of a different contribution of the two types of descriptors for the overall GEnS evaluation—giving state descriptors a higher weight, as receptors of the impacts caused by pressures. The rationale for this, as recognized by Claussen et al. (2011), is that "in principle, where GEnS for state-based descriptors (D1, 3, 4, 6) is achieved it follows that GEnS for pressure-based descriptors should also be met." This principle makes the assumption that the state eventually will reflect ceasing pressures. When the state descriptors finally reach a satisfactory level then the pressures must be having a limited (or mitigated) impact.

Visualizing and communicating the status

The outlined alternative approach also shows that concerns on integration across descriptors do not necessarily have to be a problem. There are some methods which have demonstrated that integrating the information into single values (Borja et al., 2011b), maps (HELCOM, 2010) or radar schemes (Halpern et al., 2012) is still helpful and informative for ecosystem management, despite the involved loss of information that is inherent to a single number. Information can be retained when always presenting that single number together with the main underlying data, ideally visualizing the different levels of aggregation, allowing the lookup of the status at any level and relating the status with the actual pressures that lead to the synthesized value.

As an example, the Ocean Health Index (Halpern et al., 2012) provides weighted index scores for environmental health, both a global area-weighted average and scores by country (**Figure 3**). The outer ring of the radar scheme is the maximum possible score for each goal, and a goal's score and weight (relative contribution) are represented by the petal's length and width, respectively. This way of visualizing the integration could be adapted for the MSFD, integrating at the level of region or subregion, but also showing the values within each descriptor. This would still allow managers to extract relevant information and take actions at different levels: small (or local) scale, large (regional) scale, integrative (whole ecosystem status), or for each descriptor.

Another example, applied specifically for the MSFD, using all descriptors and most of the indicators, can be consulted in Borja et al. (2011b). These authors studied a system in which the main driver for the whole area is fishing, whilst at local level some pressures such as waste discharges are important. Although the overall environmental status of the area was considered good, after the integration of all indicators and descriptors, two of the descriptors (fishing and food webs) were not in good status (**Table 5**). Interestingly, biodiversity was close to the boundary to good status (**Table 5**), suggesting that the system could be unbalanced by fishing, but affecting various biological descriptors to different degrees. This means that the pressure must be managed to avoid problems in the future, especially because the descriptors already in less than good status showed a negative trend (**Table 5**).

Hence, from the examples above and the given reasoning, both main choices are still useful: either integrate or not integrate information across descriptors. Irrespectively of which combination proposal(s) is adopted and at which level, the precautionary principle should always be followed in absence of more robust knowledge (Borja et al., 2013). As a summary, the pros and cons of each decision are shown in **Table 6**.

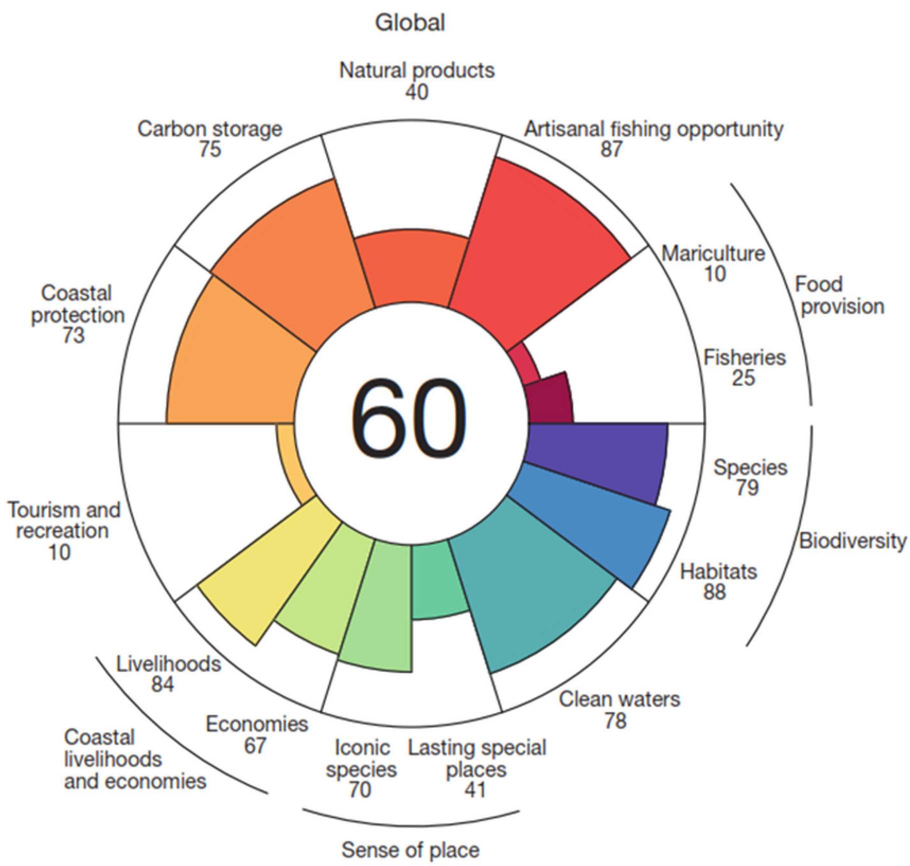

FIGURE 3 | Ocean Health Index scores (inside circle) and individual goal scores (colored petals) for global area-weighted average of all studied countries (modified from Halpern et al., 2012).

Table 5 | Example of an assessment of the environmental status, within the Marine Strategy Framework Directive, in the Basque Country offshore waters (Bay of Biscay) (modified from Borja et al., 2011b).

Qualitative descriptors	Explanation of the indicators used	Reference conditions/EQS	Recent trend	Reliability (%)	Weight (%)	EQR	Final environmental status	Final confidence ratio
Biological diversity	Integrated biological value		NA	69	15	0.51	0.08	10.35
Non-indigenous species	Ratio non-indigenous sp.	OSPAR	▲	80	10	0.98	0.10	8
Exploited fish and shellfish			▼	100	15	0.48	0.07	15
	Fishing mortality<reference			100		0.18		
	Spawning stock<reference			100		0.67		
	% large fish			100		0.59		
Marine food webs			▼	70	10	0.40	0.04	7
Human induced eutrophication		WFD	▼	94	10	0.96	0.10	9.4
	Nutrients in good status			100		0.80		
	Chlorophyll in high status			100		1.00		
	Optical properties in high status			100		1.00		
	Bloom frequency in high status			70		1.00		
	Oxygen in high status			100		1.00		
Seafloor integrity		WFD	▶	100	10	0.89	0.09	10
	Area not affected			100		0.87		
	% presence sensitive sp.			100		0.98		
	Mean M-AMBI value			100		0.83		
Alteration of hydrographical conditions			▶	100	2	1.00	0.02	2
Concentrations of contaminants	High % of sample <EQS Values are 30% of the most	WFD	▼	100	9	0.80	0.07	9
Contaminants in fish and other seafood	affected in the NEA Values are 50% of the most	WFD	▼	30	9	0.60	0.05	2.7
Marine litter	affected in Europe	OSPAR	▲	30	5	0.57	0.03	1.5
Energy and underwater noise	Moderate ship activity	OSPAR	NA	10	5	0.70	0.04	0.5
Final assessment					100		0.68 Good	75.5 High

EQS, Environmental Quality Standards; EQR, Ecological Quality Ratio, both based upon the Water Framework Directive (WFD); NA, not available; Trends: red color, negative; green color, positive (in both cases can be increasing/decreasing, depending on the indicator).

PROPOSED STEPS FOR COMBINATION

As a possible approach for the combination of assessments we propose the following steps (**Figure 4**):

- Assessments start at a low level, viz. the level of indicators and spatial scales that were defined for each specific indicator. This would result in assessment results for each indicator and each assessment area incorporating the levels of spatial assessment that was described as a nested approach (Step 1—spatial scales).
- Within one descriptor, this could result in a number of assessments for the different indicators, that all use the same scales for their assessment areas. This could be the case for descriptors like D5 and D8. In those cases, the assessments at indicator

Table 6 | Pros and cons of the decision of integrating the information across descriptors.

Procedure	Pros	Cons
No integration	Direct detection of problems (management needs) for each descriptor Useful for local managers (close to specific or local pressures) Reduces multiple accounting Easiest to implement	Does not fulfill the main aim of marine management in an integrative way Does not fully reflect the ecosystem-based approach Difficult to compare across Member States and regions
Integration (all descriptors or a subset)	Progress toward GEnS relevant at regional scale (comparable across regional seas and countries) Environmental status defined in an integrative way, as health of the ecosystem (full ecosystem-based approach) Most comprehensive approach Reflect the interlinked nature of the descriptors Easy to communicate in policy and societal domains	Loss of information on specific issues, obscuring the progress toward GEnS Can mask problems from specific descriptors/pressures May include multiple accounting May be too subjective, as it typically involves expert judgment

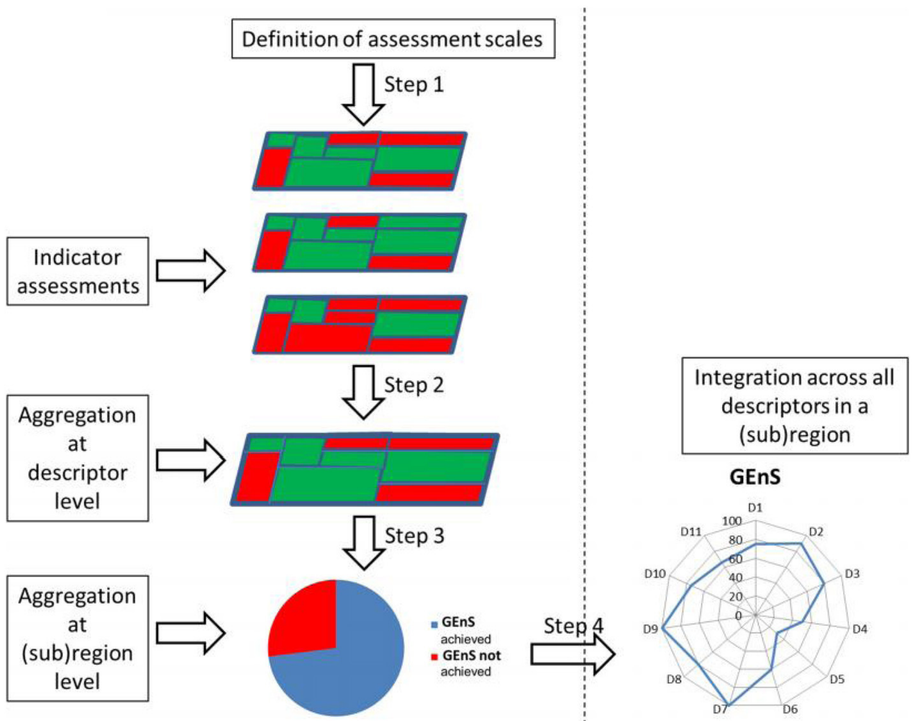

FIGURE 4 | Schematic view of steps for combination toward an assessment at subregional level. GEnS: Good Environmental Status.

level can be aggregated to assessments at descriptor level for each assessment area, using suitable aggregation rules (Step 2—aggregation within a descriptor). These steps are already commonly used procedures in OSPAR (2009) and HELCOM assessments for eutrophication and contaminants.

– For other descriptors, the spatial scales for indicators may not be the same for all indicators. This could be the case for biodiversity, where a different spatial scale may be used depending on the species or habitat. Although integration of different biodiversity components and functional groups is required, methods need further development, and a number of EU projects are focussing on this issue.

Aggregation up to this level gives a detailed assessment result that suits the information needs for identifying environmental problems and needs for measures. The result of those steps at European level would be a very high number of assessment results, for each descriptor and assessment area (comparable to presenting the WFD assessments at water body level).

The following steps could provide information at a higher level of integration presenting the required overview of the current status of the overall environmental state and the progress toward GEnS:

– Within a descriptor, the assessment results of all assessment areas within a subregion can be presented in a more integrated way (Step 3—spatial aggregation).
  - Generally, use of OOAO (if one assessment area fails GEnS, the whole subregion fails) is not useful, as it gives a very conservative result and is not informative. Also, if the pressure is highly localized this approach is not adequate, since the whole subregion could fail GEnS due to a single location (which, of course, will need specific management measures).
  - In some cases, for example if a pressure is more or less homogeneous across a whole subregion (fishing, shipping), it could be useful to apply OOAO.
– Percentage of surface area achieving GEnS: This could be a more useful approach, if the extent and intensity of a pressure can be quantified. For example, if the pressure is present in 45% of the surface area of a subregion, but the surface area not achieving GEnS is only 2%, it could be concluded that the subregion does not achieve GEnS in 2% of its area, where management measures are needed.
– Other metrics.

For some descriptors, surface area may be a good measure to express status at a subregional level: for example, D5, D8, and D10. For other descriptors, surface area is not suitable but other metrics should be considered, e.g., D1: numbers of species/habitats failing to achieve favorable conservation status; D3: number of stocks failing to meet "Maximum Sustainable Yield."

The end result of Step 3 could present the level at which GEnS is achieved at subregional scale as a pie chart. The aggregation results of Step 3 could be integrated across descriptors in a final presentation per subregion, using methods such as radar plots, or methods similar to the Ocean Health Index (Step 4—aggregation across descriptors). In this step, weighted approaches as suggested in previous sections would be considered.

CONCLUDING REMARKS

From the information provided in this overview, some conclusions can be highlighted:

– Some kind of integration across indicators, criteria and descriptors is required to arrive at assessment of GEnS or "ecosystem health."
– Integration principles should be ecologically-relevant, transparent and documented.
– Integrated assessment should not only present a classification result (primary assessment) but also address uncertainties and assess confidence of the classification result (as a secondary assessment). When carrying out an assessment at a specific scale, the decisions made in regard to integration principles/rules should be available as a sort of third assessment or backlog.
– Assessments should be planned around the question(s) to be addressed and the tool(s) to be used. Monitoring should subsequently be designed to meet the requirements of the planned assessments.
– This study provides information on combining methods to integrate ecosystem components to assess status and guidelines for scientists and managers on the steps to be followed, when deciding on assessment scales and combination approaches. Integration of taxonomic, functional and key or keystone biodiversity components into an overall biodiversity assessment able to link to GEnS and to ecosystem service provision and the sustainable management of detrimental human activities is the next challenge.

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Overview of Integrative Assessment of Marine Systems: The Ecosystem Approach in Practice

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Traditional and emerging human activities are increasingly putting pressures on marine ecosystems and impacting their ability to sustain ecological and human communities. To evaluate the health status of marine ecosystems we need a science-based, integrated Ecosystem Approach, that incorporates knowledge of ecosystem function and services provided that can be used to track how management decisions change the health of marine ecosystems. Although many methods have been developed to assess the status of single components of the ecosystem, few exist for assessing multiple ecosystem components in a holistic way. To undertake such an integrative assessment, it is necessary to understand the response of marine systems to human pressures. Hence, innovative monitoring is needed to obtain data to determine the health of large marine areas, and in an holistic way. Here we review five existing methods that address both of these needs (monitoring and assessment): the Ecosystem Health Assessment Tool; a method for the Marine Strategy Framework Directive in the Bay of Biscay; the Ocean Health Index (OHI); the Marine Biodiversity Assessment Tool, and the Nested Environmental status Assessment Tool. We have highlighted their main characteristics and analyzing their commonalities and differences, in terms of: use of the Ecosystem Approach; inclusion of multiple components in the assessment; use of reference conditions; use of integrative assessments; use of a range of values to capture the status; weighting ecosystem components when integrating; determine the uncertainty; ensure spatial and temporal comparability; use of robust monitoring approaches, and address pressures and impacts. Ultimately, for any ecosystem assessment to be effective it needs to be: transparent and repeatable and, in order to inform marine management, the results should be easy to communicate to wide audiences, including scientists, managers, and policymakers.

Keywords: assessment, integration, status, health, indicators, ecosystem approach, science-based communication

INTRODUCTION: WHY IS IT NECESSARY TO ASSESS THE STATUS OF MARINE ECOSYSTEMS?

Traditional and emerging human activities in coastal and coastal/open marine waters, including shipping, fishing, wastewater discharges, recreation, and renewable energy production, have increased greatly in recent years (OSPAR, 2009), in part due to increasing coastal populations worldwide (Halpern et al., 2015a) and the need for new resources to support that accelerated growth. Despite the benefits these activities deliver to humans, the resulting pressures, including noise, overfishing, habitat destruction, and pollution, alter marine ecosystems in a combination of synergistic and/or antagonistic ways (Crain et al., 2008; Ban et al., 2010; Piggott et al., 2015). In addition, the rapid increase in anthropogenic pressures has modified the types, frequency, extent, and duration of disturbances or impacts on aquatic species, communities, and ecosystems (Nõges et al., 2016).

Legislation at national or regional levels aims to control the potential adverse impacts of marine activities (Borja et al., 2008; Boyes and Elliott, 2014), thereby changing the paradigms of marine management from studying and managing individual pressures separately toward managing the cumulative and in-combination activities and their pressures in a holistic, ecosystem-based management approach (Agardy et al., 2011; **Box 1**). This represents one of the grand challenges in marine ecosystems ecology (Borja, 2014).

Healthy oceans provide multiple valuable ecosystem services, which in turn produce societal benefits through food provision, raw materials, energy and recreation (Costanza et al., 1997; Barbier et al., 2012; Turner et al., 2014; Turner and Schaafsma, 2015). Nevertheless, human activities can compromise the delivery of ecosystem services in the short or long term, prompting society (marine users, conservationists, policy makers, managers, and scientists) to respond. Thus ensuring that the benefits enjoyed by these stakeholders continues to rely on a scientific understanding of how various parts of the marine ecosystem are interlinked, affecting ecosystem services provision and hence human societies. Managing human activities impacting the marine environment will only be successful by undertaking a science-based integrated ecosystem approach (Agardy et al., 2011).

The Ecosystem Approach emanates from the original 12 principles defined in the Convention for Biological Diversity (CBD, 2000), which indicates that it is "a strategy for the integrated management of land," water and living resources that promotes conservation and sustainable use in an equitable way. The application of the Ecosystem Approach will help to reach a balance of the three objectives of the Convention: conservation, sustainable use and the fair and equitable sharing of the benefits arising out of the utilization of genetic resources' (CBD, 2000). In essence, this is taken to mean that the natural system structure and functioning are maintained and enhanced while at the same time the ecosystem will support human uses and deliver the ecosystem services and societal benefits required by society (Elliott, 2011). It has often been used to refer to a particular sector such as an "Ecosystem-based approach to fisheries" (Garcia et al., 2003) although the view here is that the true Ecosystem Approach cannot be sectoral but must cover all sectors. This true "Ecosystem Approach" to management requires several elements: (i) defining the source of the pressures emanating from activities; (ii) a risk assessment and risk management framework for each hazard; (iii) a vertical integration of governance structures from the local to the global; (iv) a framework of stakeholder involvement, and (v) the delivery of ecosystem services and societal benefits (Elliott, 2014). All of this may be regarded as a means of achieving both a healthy natural system and a healthy social system which is fit-for-purpose (Tett et al., 2013).

An important component of an integrated ecosystem approach to marine management is an adequate assessment of the actual environmental status, describing the health of marine ecosystems in an integrative way (Borja et al., 2013; Tett et al., 2013). Considering the spatial extent and complexity of marine ecosystems, a considerable amount of data is needed to assess the status of coastal and open seas systems with sufficient precision. For that reason cost-effective monitoring methods are needed, delivering harmonized data with an adequate spatial and temporal coverage (Borja and Elliott, 2013). To inform management planning adequately, it is especially important that assessment methods and management tools can incorporate new knowledge, new monitoring methods (to tackle the problem of covering large areas) and indicators into assessments, but still maintain comparability with previous assessments so that any change in the status can be measured and quantified.

In essence, the successful application of the Ecosystem Approach is centered around the concept of "health"—by achieving both the health of the natural, environmental system and the health of the human system (Tett et al., 2013). Health can be regarded as indicating the "fitness for survival of natural components" and maintenance of individual, population and societal well-being and so a healthy and sustainable ecosystem can also be described as one that is able to attain its full expected functioning (Costanza and Mageau, 1999). With regard to marine ecological functioning, marine monitoring should explicitly or implicitly encompass health at all levels of biological organization

BOX 1 | ECOSYSTEM APPROACH DEFINITION

The Ecosystem Approach [defined in CBD (2000)] is a management and resource planning procedure that integrates the management of human activities and their institutions with the knowledge of the functioning of ecosystems. In the management of marine ecosystems and resources, it requires to "identify and take action on influences that are critical to the health of marine ecosystems, thereby achieving sustainable use of ecosystem goods and services and maintenance of ecosystem integrity" (cf., Farmer et al., 2012, for a review of the concept of ecosystem approach in marine management). The Ecosystem Approach can be defined as the ability to fulfil the major aim of protecting and maintaining the natural structure and functioning while at the same time ensuring the creation of ecosystem services from which societal benefits can be obtained (Elliott, 2011).

(Elliott, 2011), from the health of the cell, to the tissue level, individuals of a population, populations, and communities, which is currently the most used form of ecological monitoring (Gray and Elliott, 2009; Borja et al., 2013).

In addition, as emphasized throughout all major pieces of marine governance, there is a duty to assess and ensure the health of the whole ecosystem—as ensuring protection against adverse symptoms of ecosystem pathology (Elliott, 2011; Tett et al., 2013). This allows the detection of anomalous or malfunctioning attributes as well as the ability of the ecosystem to withstand change (its resistance) and/or its ability to recover after being subjected to a marine stressor (its resilience; Borja et al., 2010b; Duarte et al., 2015).

Hence, if the marine system can produce the provisioning, regulating, cultural and supporting ecosystem services then such well-being will be guaranteed. The role of marine management then requires an ecosystem health assessment (or monitoring) programme which analyses the main processes and structural characteristics of the coupled socio-ecological ecosystem and identifies the known or potential stressors. This then requires the development of hypotheses about how those stressors may affect the ecosystem and identifies measures of environmental quality and ecosystem health to test hypotheses. Because of this we need indicators to describe the condition of ecosystem components, the extent of pressures exerted on these components and the responses to either the condition or changes to it.

Given these challenges of applying the science-based ecosystem approach which by definition integrates the natural and societal features of the system, the objective of this position paper is to review and summarize the current knowledge on the assessment of marine health status, focussing on the Ecosystem Approach. Although very many methods have been developed to assess the status of single components of the ecosystem (see a review in Birk et al., 2012), there are very few assessing multiple components to give a holistic view of the ecosystem (e.g., Borja et al., 2014).

MEASURING THE RESPONSE OF MARINE SYSTEMS TO HUMAN PRESSURES

Understanding the response of marine systems to human activities and resultant pressures requires a good conceptual basis that links the causes and consequences of change. This has been encapsulated in the DAPSI(W)R(M) approach (**Figure 1**, defined below), an improved version of the much used DPSIR approach (Wolanski and Elliott, 2015; Burdon et al., in press). This framework takes into account the different spatio-temporal scales at which **D**rivers, **A**ctivities, **P**ressures on the system, **S**tate changes, **I**mpacts (on human **W**elfare), and management **R**esponses (as **M**easures) operate. The Drivers relate to basic human needs including physiological desires, the requirement for safety and protection, employment, cultural satisfaction, or demand for goods and energy. The Impacts on human Welfare encompasses the loss of ecosystem services and employment and the psychological effects of risks and hazards. The complexity of the estuarine and coastal environment results in multiple interactions between various DAPSI(W)R(M) elements, especially in multi-use/multi-user cases. Furthermore, the nested-DAPSI(W)R(M) framework specifically recognizes the impact of *Exogenic Unmanaged Pressures* (ExUP)—such as climate change—and *Endogenic Managed Pressures* (EnMP) on

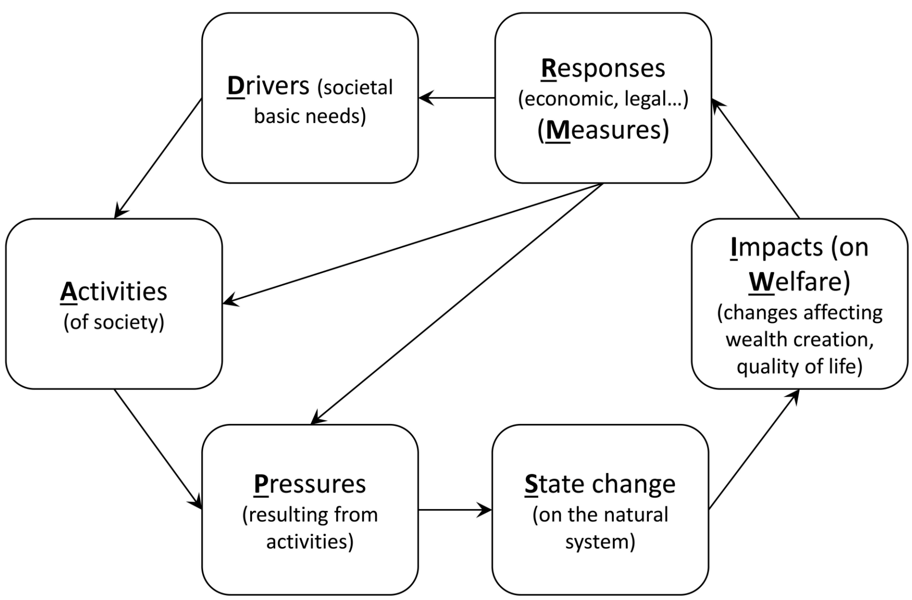

FIGURE 1 | Drivers, Activities, Pressures, State changes, Impacts on human Welfare, and management Responses as Measures [DAPSI(W)R(M)] scoping framework (Wolanski and Elliott, 2015). This management framework quantifies and assesses the Pressures, State changes, and Impacts on human Welfare but manages (using Responses as Measures) the Drivers and Activities.

the system—such as new port developments or fisheries (Elliott, 2011). This management framework quantifies and assesses the Pressures, State changes and Impacts on human Welfare but it manages (using Responses as Measures) the Drivers and Activities.

Determining the adverse effects of human activities and their resultant pressures on ecosystems is essentially a risk assessment and risk management framework (Cormier et al., 2013) that has been included in the framework of Environmental Impact Assessments (EIA) for many decades. Scientific studies of effects of single pressures on the marine environment are already well-embedded in assessments but Halpern et al. (2008) was the first to assess cumulative human activities and their potential impact at high spatial resolution. This triggered a series of national and regional studies on the effect of multiple stressors on ecosystem components (Crain et al., 2008; Ban et al., 2010; Coll et al., 2012; Korpinen et al., 2012; Micheli et al., 2013; Marcotte et al., 2015; Piggott et al., 2015; Nõges et al., 2016), with each one also aiming to improve the method and bridge caveats of the method (Halpern and Fujita, 2013).

The "cumulative impact method" itself (Halpern et al., 2008, 2015a) is a straightforward additive model linking pressures and ecosystem components over a grid of assessment cells and using expert-based weights to estimate the impacts of each pressure on specific ecosystem components (i.e., species, habitats, ecosystems). The formula is:

$$I = \sum_{i=1}^{n} \sum_{j=1}^{m} P_i \times E_j \times \mu_{i,j} \tag{1}$$

where P_i is the log-transformed and normalized value of an anthropogenic pressure in an assessment unit i, E_j is the presence or absence of an ecosystem component j (i.e., populations, species, habitats, or broad-scale habitats), and $\mu_{i,j}$ is the weight score for P_i in E_j. As the source data are high-resolution spatial layers for pressures and habitats, the scientific interest has often focused on the production of the weighing scores. As weighting scores are determined for stressor-habitat combinations, for global analyses they can miss nuanced interactions that better maps can provide, which has been done in smaller-scale assessments.

At smaller scales, weighing scores can be developed using local knowledge of system interactions, which, combined with local spatial data, has been shown to have a more significant role in the assessment results than the weighted scores in the Baltic (Korpinen et al., 2012) and the Mediterranean and Black Sea (Micheli et al., 2013). In the North Sea, Andersen et al. (2013) introduced the probability of species occurrence to the index, which is particularly suitable for highly mobile species such as seabirds, marine mammals, and big fish. With regards to pressure data, fuzzy logic was used in the U.K. sea area (Stelzenmüller et al., 2010) and in Hong Kong (Marcotte et al., 2015) to estimate the occurrence of pressures and spatial extent of adverse effects in the grid cells. In the Dutch sea area, the effects on species populations have been linked to the population demography, which allowed ecologically more realistic impact assessments (de Vries et al., 2011). When applying the index to smaller geographic scales, the need to account for the environmental variability increases. In the Finnish Archipelago Sea, a pilot study evaluated the effects of water depth and wave exposure (i.e., benthic energy) on the cumulative impacts in the index method (Sahla, 2015). The role of the two factors had significant effects on the index results in the small-scale study area.

Cumulative impacts have become a widely used element of marine assessments. For example, in Europe, the Marine Strategy Framework Directive (MSFD) particularly requires "the main cumulative and synergetic effects" to be included in Member States' assessments of Good Environmental Status (GES; European Commission, 2008). This GES should be achieved within all European seas by 2020, i.e., an area is deemed by the use of operational indicators to be one side or the other of the boundary between meeting or not-meeting GES (European Commission, 2008), using a set of 11 descriptors (biodiversity, alien species, fisheries, foodwebs, eutrophication, seafloor integrity, hydrography, pollutants in seafood and environment, litter, and noise), which encapsulate the whole ecosystem function. The European Commission (2010) proposed a set of 56 indicators to assess environmental status.

NEED OF INNOVATIVE AND COST-EFFECTIVE MONITORING

In determining the effects of pressures over large geographical scales, and taking into account the holistic view of the new integrative assessment methods, there is a clear need for developing new monitoring approaches and especially those which encompass and combine all the relevant features of ecosystems; despite this, deciding on what, where, how, when, and how often monitor is not always as obvious (Borja and Elliott, 2013). Similarly, the role of monitoring in marine management and the pros and cons of the possible monitoring framework have to be determined, including the ability of the monitoring to detect a signal of change against a background of inherent variability (the "noise" in the system; Nevin, 1969). Elliott (2011) considered 10 types of monitoring, focusing on (i) the ability to determine the overall status of an area and over a time period—this includes surveillance monitoring and condition monitoring, i.e., to monitor the features of an area and its status and then *a posteriori* to detect a trend; (ii) the ability to determine whether an area or a time period meets a pre-determined and pre-agreed status such as a baseline, threshold, or trigger value, which may be defined in law or in licence conditions and hence *a priori* has the status defined—this includes compliance monitoring and operational monitoring, and (iii) once a difference has been detected between what is expected and what is found, i.e., change has occurred, then that sequence or trajectory of change, and its causes and consequences have to be determined—this requires investigative or diagnostic monitoring and possibly feedback monitoring and toxicity analyses in which the assessment has a direct and real-time link to management.

Taking this into account, here we summarize and focus on four main promising approaches, which can assist monitoring, with importance in marine systems: genomic tools, remote sensing, acoustic devices, and modeling, which can be combined in a novel way to cover the needs of monitoring large geographical areas.

Genomic tools are seen as a promising and emerging avenue to improve ecosystem monitoring, as these approaches have the potential to provide new, more accurate, and cost-effective measures. Several techniques have been identified as potential substitutes of traditional approaches for various applications (Bourlat et al., 2013), and some can even provide measurements that were not possible before the genomic era (**Figure 2**).

Meta-omic (metabarcoding, metagenomics, and metatranscriptomics) techniques are particularly appealing as they allow the analysis of environmental samples without the need to isolate organisms. Probably, the most promising, developed, and straight-forward genomic tool for environmental monitoring is metabarcoding (Cristescu, 2014; Chariton et al., 2015). This technique consists of taxonomically identifying the organisms present in a given sample based on a small DNA fragment (called a "barcode") that is unique to each species. Potential applications of metabarcoding in marine monitoring include calculating biotic indices based on taxonomic composition, detection of invasive species or understanding trophic interactions by analysing fecal samples or stomach contents (Aylagas et al., 2014; Chariton et al., 2015; Dafforn et al., 2015). However, the routine application of this technique still requires that standardized practices at each step of the procedure are developed. For example, sampling strategies, nature of the barcode selected, conditions of barcode amplification or available reference barcode library may affect the taxonomic composition inferred from genomic data (Aylagas et al., 2014). Several campaigns of sampling standardization have already been initiated, such as the Ocean Sampling Day (Kopf et al., 2015) for marine microbe sampling, and the use of Autonomous Reef Monitoring Structures (ARMS; http://www.pifsc.noaa.gov/cred/survey_methods/arms/overview.php) for sampling both prokaryotic and eukaryotic organisms. There is therefore an urgent need to compare both traditional and molecular based taxonomic composition inferences so that metabarcoding can be introduced as a regular tool in monitoring programs.

Satellite remote sensing is another promising monitoring approach. Although this has long been used to monitor chlorophyll *a* (Coppini et al., 2012), it has only recently been applied to determine phytoplankton size structure (Barnes et al., 2011; Brewin et al., 2011), composition and functionality (Moisan et al., 2013; Palacz et al., 2013; Rousseaux et al., 2013) and monitoring of harmful algal blooms (Frolov et al., 2013). However, there are still few studies which assess the ecological status of coastal an d open marine waters based on the phytoplankton component (Gohin et al., 2008; Novoa et al., 2012), thus requiring the development in support of assessments in large marine areas.

Acoustic devices are a monitoring approach built on the traditional use of benthic habitat mapping (see Brown et al., 2011), that can be used to determine the composition and abundance of different biodiversity components, especially fish and cetaceans (André et al., 2011; Denes et al., 2014; Fujioka et al., 2014; Parks et al., 2014). Again, there are few studies regarding the use of underwater acoustics to assess the status of diverse ecosystem components and indicators (Trenkel et al., 2011).

Furthermore, certain types of modeling provide a valuable accompanying approach to monitoring, for example to increase spatial coverage of environmental variables and predict spatial distribution patterns of different ecosystem components, i.e., through species distribution modeling (Reiss et al., 2015). Deterministic models can be used to predict physico-chemical characteristics such as water quality parameters or fish stock size,

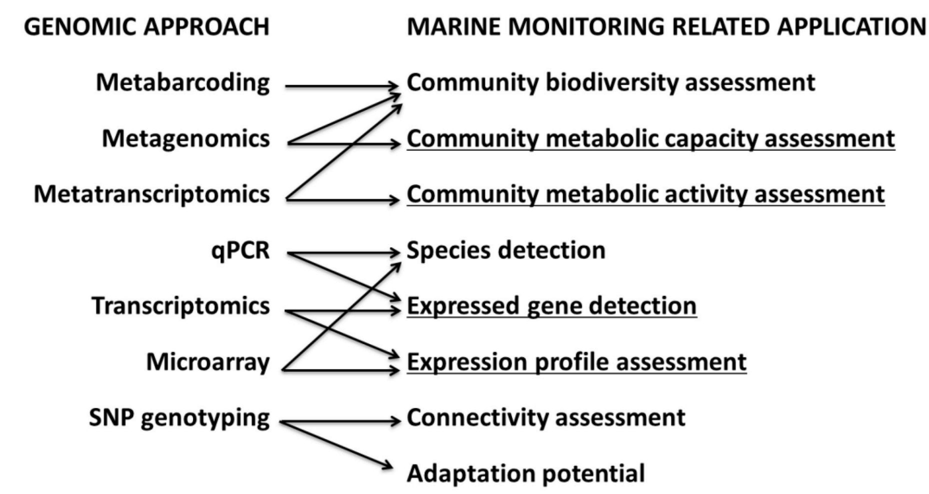

FIGURE 2 | Genomic approaches (left) and their potential marine potential application (right). Metabarcoding, metagenomics, and metatranscriptomics consist respectively on sequencing a region of the genome, the genome or the transcriptome of a whole community; qPCR (quantitative PCR) and microarrays consist on measuring the quantity of DNA or RNA in a given sample at low and high throughput respectively; SNP genotyping consists on determining the genotype of selected Single Nucleotide Polymorphisms of individuals from the same species in order to estimate differences in allele frequencies among populations. Applications that cannot be performed using traditional techniques are underlined.

whereas empirical models are valuable to link species presence to habitat characteristics and thus extrapolate from a monitored area to the wider spatial coverage (Groeneveld et al., in press; Peck et al., in press). Ecological modeling is being used to describe or understand ecosystem processes, and is currently a valuable approach used to predict and understand the consequences of anthropogenic and climate-driven changes in the natural environment (Piroddi et al., 2015). Piroddi et al. (2015) have reviewed the most commonly used capabilities of the modeling community to provide information about indicators used to assess the status in marine waters, particularly on biodiversity, food webs, non-indigenous species and seafloor integrity. Ecosystem modeling has the potential to show the complex, integrative ecosystem dimensions while addressing ecosystem fundamental properties, such as interactions between structural components and ecosystem services provided (Groeneveld et al., in press). As such, some modeling tools (i.e., species distribution modeling) can be used in support of monitoring to predict the distribution of species in areas not monitored or to derive indicators in support of the assessment process.

Traditional monitoring tools (i.e., direct sampling, visual identification, etc.) and these new monitoring approaches are producing information to generate the indicators needed to assess the status of marine systems, as presented below.

EXAMPLES OF HEALTH AND STATUS ASSESSMENT IN MARINE SYSTEMS

The following sub-sections give examples (in chronological order of publication) of integrative assessment methods. All can be applied to large marine areas in open and coastal waters. Most of the methods are motivated by international legislation or conventions and use various indicators to derive the status assessment. The most important differences are their choice of indicators and the way these are synthesized into the overall ecosystem health. **Table 1** summarizes the main characteristics of the methods described here.

Ecosystem Health Assessment Tool

With the adoption of the HELCOM (Baltic Marine Environment Protection Commission - Helsinki Commission) Baltic Sea Action Plan, the Contracting Parties to the Helsinki Convention launched an ambitious Action Plan to restore ecosystem health of the Baltic Sea (HELCOM, 2007). As the Action Plan is based on the Ecosystem Approach, tracking, and documenting progress in meeting the vision and objectives was required. Hence, a plan for establishing a region-wide baseline was developed and implemented through the production and publication of an indicator-based assessment of ecosystem health in the Baltic Sea region (HELCOM, 2010a).

The ecosystem health is based on a Baltic-wide application of a multi-metric indicator-based assessment tool, the HELCOM Ecosystem Health Assessment Tool (HOLAS; HELCOM, 2010a). This is based on existing HELCOM tools for assessing "eutrophication status" (HEAT; HELCOM, 2009a and Andersen et al., 2010, 2011), "biodiversity status," (BEAT; HELCOM, 2009b and Andersen et al., 2014) and "chemical status" (CHASE; HELCOM, 2010b; Andersen et al., 2016). Currently, the HOLAS tool is under revision to ensure applicability for the MSFD assessments in the future. This will include revision of the aggregation rules for the indicators that have been developed and agreed in the HELCOM CORESET project (HELCOM, 2013) where the jointly agreed set of indicators is to finalized currently.

Three dilemmas were faced. First, using few groups of indicators (one or two) and averaging across many indicators may potentially lead to "thinning" and potentially to "upward"

TABLE 1 | Summary of the main characteristics of the methods described here.

Characteristics of the methods	Methods described				
	HOLAS	**No name**	**OHI**	**MARMONI**	**NEAT**
References	HELCOM, 2010a	Borja et al., 2011	Halpern et al., 2012, 2015a	www.sea.ee/marmoni	www.devotes-project.eu
Application area	Baltic Sea	Bay of Biscay	Global and at 11 smaller scales	Baltic Sea	European Seas
Associated legislation	HELCOM	MSFD	None at global scale, various national and international at smaller scales	HELCOM and MSFD	MSFD
Required input info	HELCOM indicators	MSFD indicators and descriptors	Indicators and goals	HELCOM or MSFD indicators	MSFD indicators
Weighting	Yes	Yes	Yes	Yes	Yes
Aggregation	OOAO	Mean	Mean	Mean	Mean, but others possible
Reference conditions	Yes[a]	Yes	Yes	Yes	Yes
Scale of result	0–1 and 0–∞	0–1	0–100	0–100	0–1
Status classification levels	5	2	2	2	2 to 5
Uncertainty	Yes	Yes	In developments	Yes, qualitative	Yes, quantitative

For the complete names of the methods, see text. MSFD, Marine Strategy Framework Directive, HELCOM, Helsinki Convention; OOAO, One out, all out.
[a]*For contaminants, target values are used instead of background values/reference conditions.*

misclassification (i.e., arriving at a better status classification compared to the use of more groups; lessons learned from the development of the CHASE prototype tool). Second, many groups of indicators and stringent use of the "one out, all out" principle, in which overall status of a region defaults to the status of the worst biological component (Hering et al., 2010), may potentially lead to "downward" misclassifications (i.e., arriving at a poor status classification compared to the use of fewer groups; lessons learned from HEAT and Borja and Rodríguez, 2010). The one-out-all-out principle has been adopted in the European Water Framework Directive (WFD; European Commission, 2000). Third, in some cases, good indicators and target values do not yet exist.

The HOLAS tool has four steps (**Figure 3**). In step 1, indicators are nested in three categories (CI: biology; CII: chemistry; CIII: supporting). In step 2, either an Ecological Quality Ratio (EQR) or a Chemical Score (CSchem) is calculated. For categories I and III, a weighted average Ecological Quality Ratio (EQRbio and EQRsupp; see Equation 2) is calculated (ranging from 0, bad status, to 1, high status, *sensu* the WFD, European Commission, 2000) and for category II, a Chemical Score (CSchem; see Equations 3 and 4) is calculated as the ratio of the status against a threshold value. In step 3, categories I, II, and III are classified in five classes (High, 0.0–0.5; Good, 0.5–1.0; Moderate, 1.0–5.0; Poor, 5.0–10.0; and Bad > 10.0). Finally, in step 4, category classifications are combined (using the lowest ranking classification cf. the "one out, all out" principle (see Borja and Rodríguez, 2010), into a final classification of "ecosystem health" (in 5 classes).

The applied assessment principles differ for category I and II indicators. For category II indicators, as well as category III, the assessment principles on the indicator level is straight-forward, the only difference relate to whether the response is numerically positive or negative to an increase in pressure:

$$\begin{aligned} \text{EQR} &= \text{RefCon/Obs} \qquad \text{(positive response)} \\ &= \text{Obs/RefCon} \qquad \text{(negative response)} \end{aligned} \tag{2}$$

where RefCon is the reference condition and Obs is the observed value. Detailed descriptions of the above principles as well as integration principles within groups of indicators can be found in HELCOM (2010a) and Andersen et al. (2010, 2011, 2014).

For category II indicators each indicator is simply assessed against a threshold level by calculating the ratio and the results of the indicators are then combined to obtain the status for each element. For each of the indicators (n) in an assessment unit (i.e., a spatial quadratic unit), the Contamination Ratio (CR) of the measured concentration (C_m) to a relevant assessment criterion for GES ($C_{Threshold}$) is calculated using:

$$CR = \frac{C_m}{C_{Threshold}} \tag{3}$$

Integration of the CRs of the indicators is calculated as a Contamination Score (CS; Equation 4):

$$CS = \frac{1}{\sqrt{n}} \sum_{i=1}^{n} CR_i \tag{4}$$

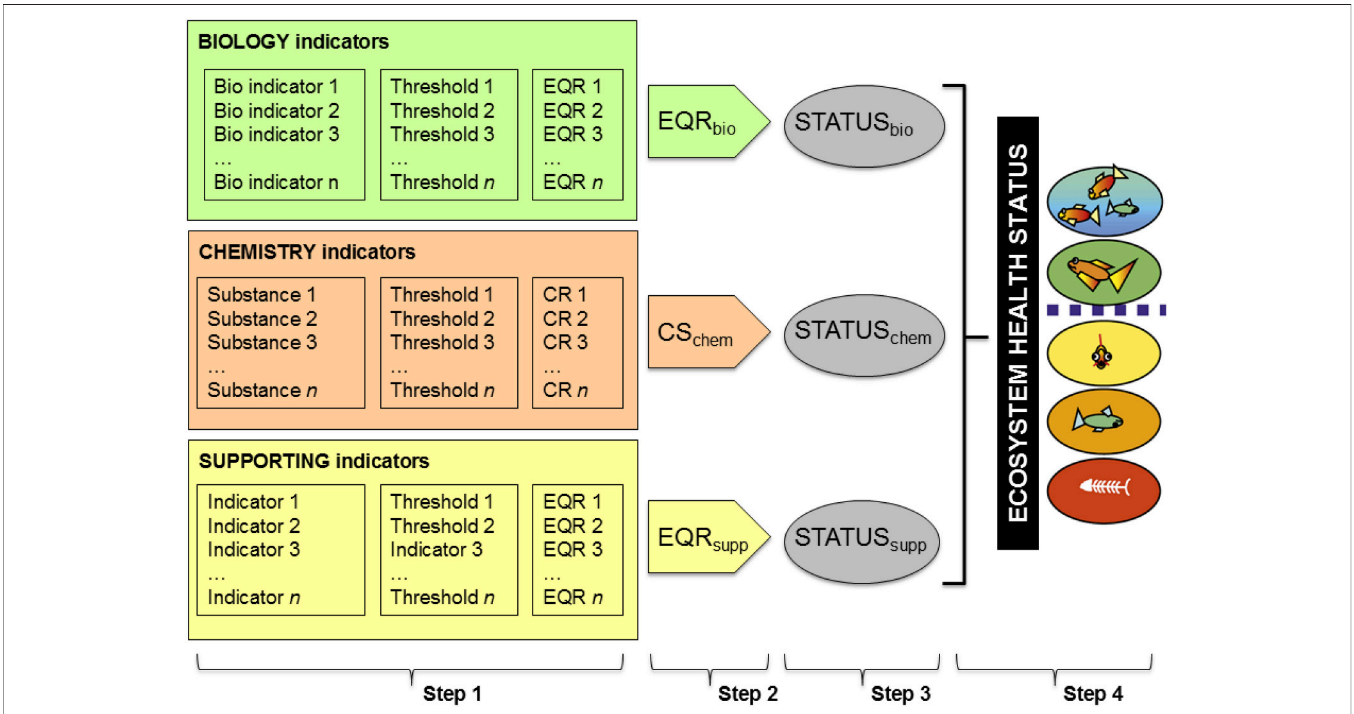

FIGURE 3 | Conceptual model of the HELCOM HOLAS tool. Indicators used for thematic assessment are integrated by weighted averaging in three categories (steps 1 and 2), and the score-based classifications (step 3) are further integrated by the "One Out-All Out" principle (step 4). The fish represent the five status classes: high (blue), good (green), moderate (yellow), poor (orange), and bad (red) (see text and Andersen et al., 2014 for further details). EQR, Ecological Quality Ratio.

A detailed description of these assessment principles and calculations as well as their practical use can be found in Andersen et al. (2016). As such, the HOLAS tools has been tested and applied in the Baltic Sea (HELCOM, 2010a) for the classification of ecosystem health status in selected open and coastal waters (**Figure 4**).

A Method for the Marine Strategy Framework Directive, Within the Bay of Biscay

The first attempt for assessing status according to the MSFD, using the 56 indicators proposed by the European Commission (2010), was undertaken in the southern Bay of Biscay (Borja et al., 2011). The approach was based on combining indicators, by grouping the marine ecosystem components into four distinct and interlinked systems: (i) water and sediment physico-chemical quality (including general conditions and contaminants); (ii) planktonic (phyto- and zoo-plankton); (iii) mobile species (fishes, sea mammals, seabirds, etc.), and (iv) benthic species and habitats. These ecosystem components, affected by different human pressures, are linked to the 11 MSFD descriptors and, as such, indicating the quality of the different indicators (see Borja et al., 2010a, 2011).

Borja et al. (2011) assessed each indicator and descriptor by deriving an EQR (as in the WFD and the HOLAS method, see Section Ecosystem Health Assessment Tool) in which monitoring data are compared with reference conditions of each indicator, a fundamental step in any quality status assessment (Borja et al., 2012).

After calculating a status value for each of the indicators, the method integrates the values at the level of single descriptors and then combines all 11 descriptors into a final assessment (**Table 2**). Weighting each descriptor has been proposed, and could depend on its relationships with dominant pressures in the study area. Weighting would thus emphasize certain descriptors, e.g., fishing in **Table 2** (see also recommendations by Borja et al., 2010a).

An environmental status value was derived by multiplying the weight by the EQR of each descriptor and dividing by 100, and an overall environmental status value was obtained by adding all the values for each descriptor. The indicators and descriptors that have values below GES (see Section Measuring the Response of Marine Systems to Human Pressures) require management action and can be easily identified (**Table 2**). Criteria for achieving GES can be found in Rice et al. (2012), Borja et al. (2013), and ICES (2013). The method also assesses the reliability of the result in a qualitative way, taking into account data availability and confidence in the methods used in assessing the status, and following the same approach as for the assessment.

Ocean Health Index

The Ocean Health Index (OHI; Halpern et al., 2012) was a logical progression following the development of the cumulative impacts framework (Halpern et al., 2008), as the OHI includes not only the negative impacts exerted on the oceans but also captures the tangible and less-tangible benefits derived from the oceans. The OHI framework scores a suite of benefits ("goals") that are delivered to people by assessing the current status and likely future state (including pressures and resilience measures) of each goal for each region that together comprise the whole assessment area (**Figure 5**). A single OHI Index score is calculated by

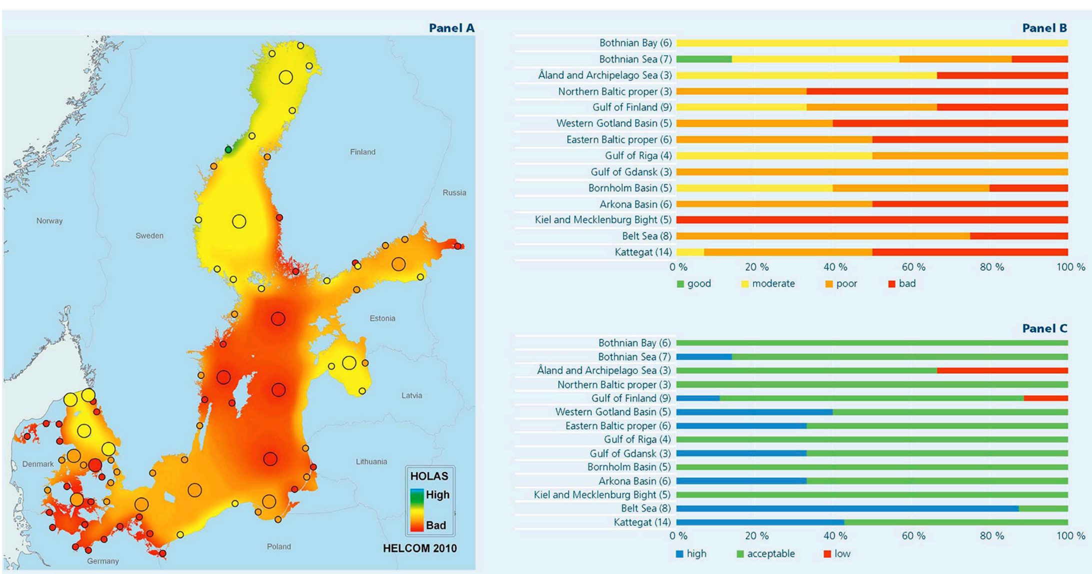

FIGURE 4 | Classification of "ecosystem health status" in the Baltic Sea. In panel **(A)**, classifications are spatially interpolated in order to illustrate that the impairment is a large scale problem. Panel **(B)** shown classification per sub-region [expressed as good (green), poor (yellow), poor (orange), or bad (red)], while panel **(C)** shows the confidence assessment of the classifications per sub-region [expressed as a high confidence (blue), a moderate but acceptable confidence (green), and a low confidence (red)]. See HELCOM (2010a) for details.

TABLE 2 | Example of an assessment of the environmental status, within the Marine Strategy Framework Directive, in the Bay of Biscay (modified from Borja et al., 2011).

MSFD Descriptor	Indicators used	Reference conditions	Reliability (%)	Weight (%)	EQR	Final environmental status	Final confidence ratio
1. Biological diversity	Integrated biological value		69	15	0.51	0.08	10.35
2. Non-indigenous species	Ratio non-indigenous sp.	OSPAR	80	10	0.98	0.10	8
3. Exploited fish and shellfish			100	15	0.48	0.07	15
	Fishing mortality < reference		100		0.18		
	Spawning stock < reference		100		0.67		
	% large fish		100		0.59		
4. Marine food webs			70	10	0.40	0.04	7
5. Human-induced eutrophication		WFD	94	10	0.96	0.10	9.4
	Nutrients in good status		100		0.80		
	Chlorophyll in high status		100		1.00		
	Optical properties in high status		100		1.00		
	Bloom frequency in high status		70		1.00		
	Oxygen in high status		100		1.00		
6. Seafloor integrity		WFD	100	10	0.89	0.09	10
	Area not affected		100		0.87		
	% presence sensitive sp.		100		0.98		
	Mean M-AMBI value		100		0.83		
7. Alteration of hydrographical conditions			100	2	1.00	0.02	2
8. Concentrations of contaminants	High % of samples < Standard	WFD	100	9	0.80	0.07	9
9. Contaminants in fish and other seafood	Values are 30% of the most affected in the NEA	WFD	30	9	0.60	0.05	2.7
10. Marine litter	Values are 50% of the most affected in Europe	OSPAR	30	5	0.57	0.03	1.5
11. Energy and underwater noise	Moderate ship activity	OSPAR	10	5	0.70	0.04	0.5
Final assessment				100		0.68 Good	75.5 High

EQR, Ecological Quality Ratio; WFD, Water Framework Directive; MSFD, Marine Strategy Framework Directive; OSPAR, Oslo-Paris Convention; NEA, North-East Atlantic; M-AMBI, multivariate-AMBI; Green, good status; Red, less than good status. Yellow color show the values for indicators included within several descriptors (in blue).

combining all goal scores with the following equation:

$$I = \sum_{i=1}^{N} \alpha_i I_i,$$

where $I_{1\ldots N}$ are the n goal scores and α_i are the goal weightings (equal by default although can reflect relative importance of goals within the assessment area). Individual goal (and sub-goal) scores I_i are based on the current status relative to its reference state along with the recent trend in status and the interaction of pressures and resilience measures. Assessments to date have generally evaluated 10 goals, some of which have sub-goals.

The framework can be used to assess areas with different spatial scales, characteristics and priorities as it is tailored to the specific context, such that only relevant goals are assessed. Furthermore, scores are calculated relative to reference points based on what is important within the assessment area. OHI assessments use existing information so that assessments reflect the best available knowledge of the system at the time of the assessment; this can require indirect measures to be included in assessments where the direct measures that ideally would be included are unavailable. Therefore, assessments not only produce scores that can be used to inform policy decisions, but they also identify knowledge gaps that can also be highly valuable to prioritizing further management action.

To date, 11 assessments have been completed for seven different locations: globally for all coastal nations and territories for each year 2012–2015 (Halpern et al., 2012, 2015b), Brazilian coastal states (Elfes et al., 2014), the U.S. West Coast states and sub-states (Halpern et al., 2014), Fiji (Selig et al., 2015), Israeli Mediterranean districts (Tsemel et al., 2014), Canada (in prep), Ecuador Gulf of Guayaquil (in prep), and Chinese coastal provinces (in prep). Because the global assessment has been repeated annually for 4 years (Halpern et al., 2012, 2015b; www.ohi-science.org), emerging trends and patterns in calculated scores are becoming apparent. For example, continued improvement in the global economy since the economic collapse of 2008 is reflected in improving coastal livelihoods and economy scores, and the steady increase in creating marine protected areas

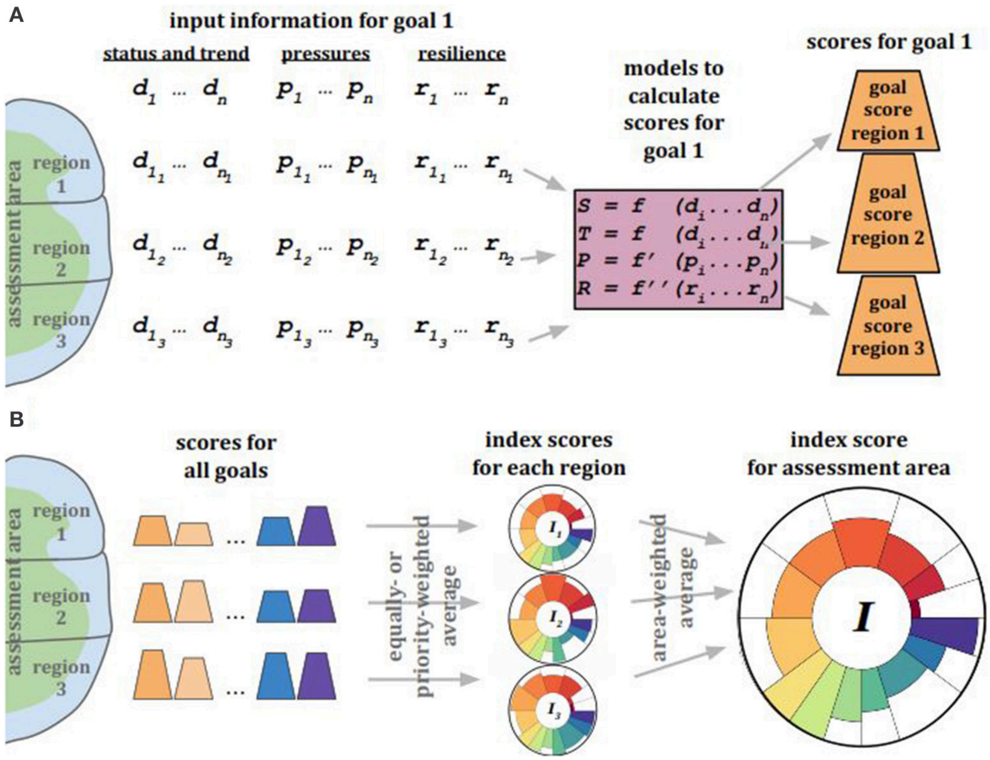

FIGURE 5 | Ocean Health Index scores are calculated for each goal and for each spatially defined and non-overlapping region within the assessment area. (A) To calculate scores for a single goal, the best locally available information for each region is used for status and trend (d), pressures (p), and resilience (r). This information is used in mathematical models to calculate the status (S) and trend (T) of that goal, as well as the pressures (P) and resilience (R) relevant to that goal. S, T, P, and R are to calculate a score for each goal for each region. This process is done for all goals and sub-goals in the assessment framework. **(B)** After all scores are calculated for all goals for every region, those goal scores are combined with equal weighting (by default; unequal weighting based on context-specific priorities is possible) for each region to produce an Index score for each region, and finally for the entire assessment area.

worldwide has increased part of the sense of place goal. Repeated assessments also incorporate newly available data (e.g., when new satellites are launched creating a new data source), and can be used to evaluate if or how well particular policy actions are performing in changing ocean health. But to be relevant for policy, assessments should be conducted at governance scales appropriate for management action. At a minimum, this usually requires assessments at the regional sea or national scale.

The OHI framework was first applied in two countries of highly different sizes, both relatively information-limited: Brazil and Fiji. In each case, it was found that individual goal models could be redeveloped or improved with at least some local information, while relying on inputs and models from global assessments for goals where such information was unavailable (Elfes et al., 2014; Selig et al., 2015). The framework was also applied to a data-rich setting, the U.S. West Coast assessment. In this case high resolution and quality data were available for nearly all goals and data components of the Index. Regionally-appropriate reference points for some goals were also developed, allowing the assessment to better reflect region-specific preferences within the assessment area (Halpern et al., 2014).

Completion of the 11 assessments noted above as well as involvement in additional ongoing assessments has allowed refining and improving conceptual and technical aspects of the tools and resources available to conduct an OHI assessment (Lowndes et al., 2015). Computational and visual tools as well as instructions for their use have been developed, and these tools are shared and support is given with independent assessment efforts. As with the cumulative human impacts framework (Halpern et al., 2008), the OHI framework has also triggered independent groups to assess areas of interest using local input information representing local characteristics and priorities. Of the 11 completed OHI assessments, four have been independently-led. The first was led by the Israeli National Nature Assessment Program HaMaarag, assessing the Israeli Mediterranean coast and incorporating local measures, including tourism patterns and desalinated water and setting reference points based on local priorities (Tsemel et al., 2014). At the same time, a group funded by the Canada Healthy Oceans Network (CHONe) completed a feasibility study where they added attributes important to Canada and recalculated scores with methods from the global assessment. They also led a survey on how goals should be weighted and will be able to build from this initial work and calculate scores separately for each Canadian ocean. The most recently completed assessments were led by the governments of Ecuador and China. These assessments were able to use government statistics as input information and management targets as reference points for many goals. Additional independent assessments are also currently underway, in Spain, the Baltic Sea, Chile, Colombia, the Arctic, Hawaii, Peru and British Columbia.

Each OHI assessment can build from past assessments, conceptually and technically, since all data, methods and code are freely available online (www.ohi-science.org). Such transparency allows interrogating methods and results, but perhaps most importantly facilitates repeated assessments within a given area, allowing managers, scientists, and stakeholders to track and compare scores through time. A single assessment provides an important baseline of overall ocean health and guidance on strategic actions to improve ocean health; repeated assessments allow determining the efficacy of management measures taken.

MARMONI Tool

The MSFD Marine Biodiversity Assessment Tool (referred to as the MARMONI Tool) is a publicly available web-based application developed in the framework of the LIFE+ MARMONI project with the aim to perform MSFD compatible, indicator-based, integrated marine biodiversity assessment (www.sea.ee/marmoni/). It uses various indicators for the assessment area with several options for GES determination (see also Section A Method for the Marine Strategy Framework Directive, within the Bay of Biscay). The boundary value, determining when GES is attained, can be defined as a fixed value or an interval of values or through an acceptable deviation (value or percent) from reference condition, GES can also be defined as a direction of trend or by expert judgment (Auniņš and Martin, 2015).

The MARMONI tool follows a hierarchical approach (**Figure 6**). The first level is the assessment of the operational indicators according to their specific methodology (indicator specific assessment methods including: either GES is defined through reference conditions and acceptable deviation or GES is defined by a range of values or GES is defined by trend direction), resulting in attributing either GES or non-GES status. The tool uses a binary approach where an indicator reaching GES is scored 100, while an indicator which does not reach GES is scored 0. The second level constitutes the aggregation of assessment results to each Commission Decision (CommDec) indicator (e.g., distributional range, distributional pattern, habitat area; European Commission, 2010). This is carried out by calculating the mean of individual indicator scores within each aggregation unit. The next aggregation is at CommDec criteria level (e.g., species distribution, population size, and habitat extent) followed by a final aggregation at descriptor level (biodiversity in this case; European Commission, 2010). The method includes the possibility of weighting different indicators in three classes and the ability to test different scenarios by excluding different indicators entered in the database for scenario testing.

A separate procedure is performed to estimate the uncertainty of the assessment across four different elements: (i) spatial

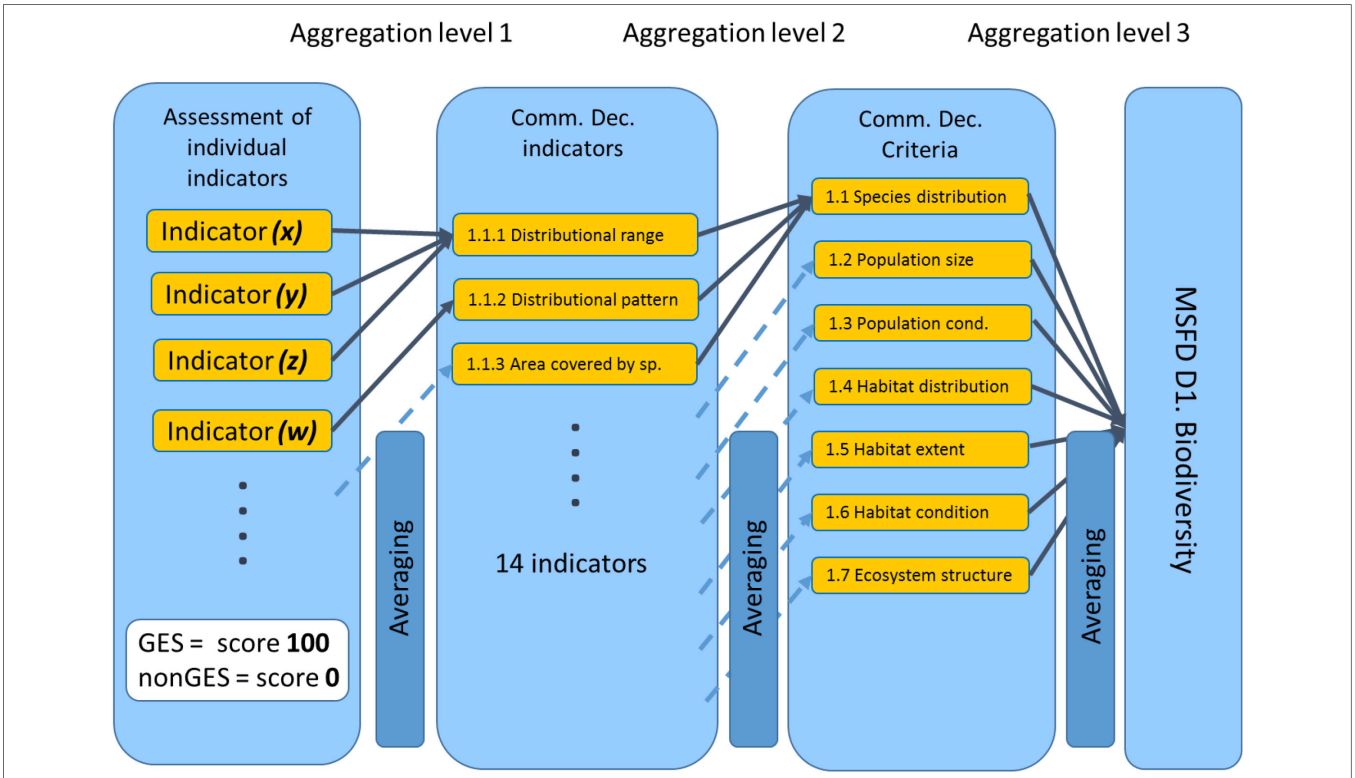

FIGURE 6 | MARMONI hierarchical approach for aggregation of assessment scores from individual indicators, through different levels used in Commission Decision document (European Commission, 2010). GES, Good Environmental Status; MSFD, Marine Strategy Framework Directive; D1, Descriptor 1.

uncertainty; (ii) temporal uncertainty; (iii) uncertainty associated with the measurement of operational indicator, and (iv) uncertainty associated with defining its GES level or targets. The spatial representation aims to describe how well the data used for the indicator calculation cover the area of interest, whether the sampling is complete in terms of spatial coverage and whether all relevant habitats are well covered. Uncertainty connected to temporal aspects can come from different sources of temporal variability (i.e., within year or assessment season, seasonal variability and between year variability) as relevant. To assess the confidence level at each level of temporal resolution, a measure of variance needs to be calculated. The quality of assessment data depends on whether the indicator values are entirely based on objective measurements, subjective estimations or modeled indicator values. Uncertainty is low when the GES boundary or target is based on robust historical data. Each of these uncertainty elements is attributed to one of three uncertainty classes. At each level of aggregation the median of the uncertainty elements is calculated and presented on each level in the same way as the assessment score.

The tool displays information about assessment scores at Descriptor and CommDec criteria levels, the number of operational indicators for different CommDec criteria and indicators, the biological features that are covered by indicators and the source of the greatest gaps, and the overall uncertainty class at each assessment level (**Figure 7**). Although the resulting assessment is intended as a basis for drawing conclusions on whether the assessed area has achieved GES or not, there are no strict MSFD guidelines on this kind of decision (e.g., how many or what proportion of the indicators not being in GES are allowed, for the area to still be considered being in GES). The tool is designed to illustrate on how far is the study area away from achieving GES for all indicators/criteria and where are the gaps in monitoring rather than to provide an unambiguous answer to whether an area is in GES or not. This is further complicated by the fact that Member States have not yet decided on the aggregation rules for combining the assessments based on individual descriptors (Borja et al., 2014).

The MARMONI tool has been tested on data from four areas within the Baltic Sea (Martin et al., 2015) and shows that it is an easy-to-use and straightforward method to perform assessment of the status of MSFD Descriptor 1 (biodiversity). The main limitations for the practical application can be the lack of operational indicators and data covering different biodiversity components of the assessment area. Using more operational indicators as well the even distribution of them between different biodiversity components and assessment criteria will increase the confidence of the assessment result.

NEAT (Nested Environmental Status Assessment Tool)

This is a tool developed by the DEVOTES project (http://www.devotes-project.eu, based on Andersen et al., 2014) for assessing the environmental status of marine waters, within the European MSFD (European Commission, 2008). It focuses on

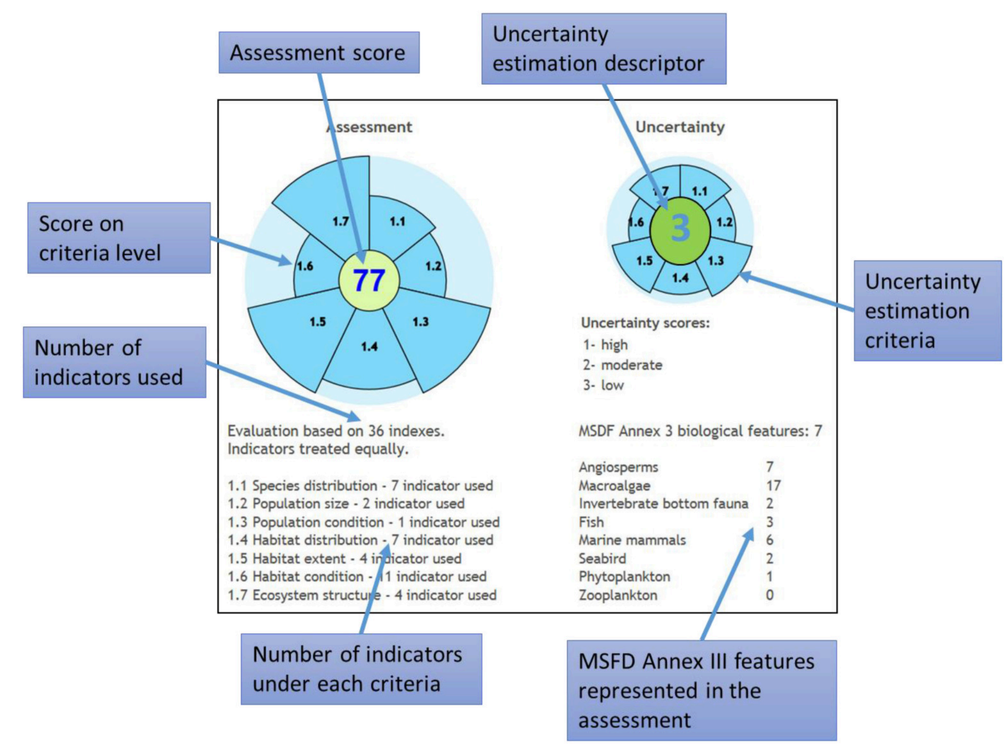

FIGURE 7 | Information displayed at result screen of MARMONI biodiversity assessment tool. MSFD, Marine Strategy Framework Directive.

biodiversity status rather than the pressures leading to state changes. The indicators are thematically grouped, assigning them to the corresponding habitats, biodiversity components, spatially defined marine areas and pressures for which they are used (available as the DEVOTool software; Teixeira et al., 2014). This can be used to check for a suitable set of indicators in terms of coverage of all important biodiversity components and habitats within assessment. As NEAT is designed around the Ecosystem Approach (Tett et al., 2013), encompassing all ecosystem features relevant to the assessment (Gray and Elliott, 2009) can thus be safeguarded.

NEAT guides a user through the assessment process once the user defines the spatial scope of the assessment. This can be a regional sea or any other number of geographical entities and is based on Spatial Assessment Units (SAU). Since biodiversity is rooted in the spatial domain (without space there is no biodiversity; Sarkar and Margules, 2002), the indicators are assigned to a SAU and a habitat. To do this, multiple hierarchically nested SAUs can be used in one assessment and different indicators can be used for each of them. The tool includes a nested hierarchy of habitats from which to choose and each of the SAUs used in an assessment will thus be assigned to corresponding habitats. The combination of SAU and habitat then determines which indicators can be used in the chosen setting (**Figure 8**). Every indicator used in the tool also carries information on the numerical scale of its status classification (number of status classes, class boundary values).

The next step is to enter the observed indicator values for different combinations of SAUs and habitats. Indicator values are entered alongside with their classification scale. Before employing these values in the assessment calculation, they are mathematically transformed to a common normalized numerical scale (from 0 to 1). Furthermore, together with the indicator values, a value or judgment on their standard error must also to be entered to allow an integrated uncertainty assessment.

NEAT uses weighting factors in the assessment calculation but, in contrast to other tools, it does not weight the indicators. Instead, the weighting is done on the entities of interest, namely the important features of the ecosystem such as the SAUs, habitats or biodiversity components. By default, all SAUs are weighted equally but SAUs within the assessment may be weighted differently in order to emphasize the importance of specific parts of the whole assessment area. For this, the SAUs can be weighted using their area and/or by their quality giving, for example, the relative value of SAUs, a feature of the assessment as quality is an assessment criterion. Further, habitats can also be weighted by either their area or their quality.

Essentially, the final assessment value is calculated as a weighted average, where the final weights are combined with the observed indicator values. In this simple example of synthesis, no special rules are applied but the tool design allows assigning different aggregation rules at the various steps in the calculation of the overall assessment value. As an example, instead of using the default algorithm, specific needs may require to employ the one-out-all-out principle between partial results of the weighted indicator values.

In order to assess the uncertainty in the final assessment value and thus the uncertainty of the biodiversity state classification, the standard error of every observed indicator value is used. The observed value is assumed to represent the mean value of a normal distribution with the standard error being its standard deviation. The resulting probability distribution is used to run a simulated assessment using the Monte-Carlo technique with 10,000 iterations. During each iteration the indicator values are picked randomly from the given probability distributions and the final assessment value is calculated. The 10,000 realizations integrate the uncertainty of the overall status assessment and can be displayed as a histogram of simulation results falling into the various status classes.

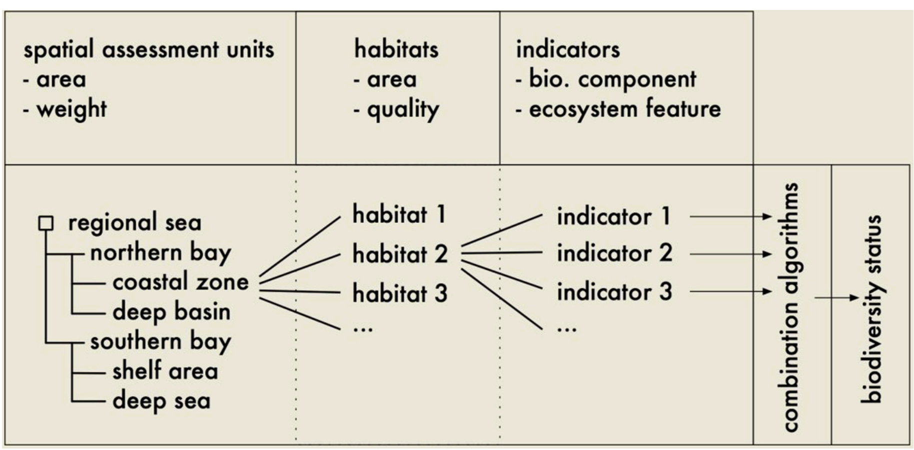

FIGURE 8 | Conceptual model of the design of the Nested Environmental Assessment Tool (NEAT). Every Spatial Assessment Unit (SAU) may be assigned to several habitats, every SAU/habitat combination to several indicators. SAUs and habitats are characterized by their area and a weight/quality while indicators are assigned to biodiversity components or other ecosystem features. The subsequent algorithms combine the indicator values using the weighting of their corresponding SAUs and habitats and result in the overall biodiversity status.

LESSONS LEARNED FROM COMPARING THE TOOLS

This review summarizes key attributes of some of the main tools and approaches currently available as an illustration of the means of assessing marine waters under an Ecosystem Approach. Such assessment relies on our ability to determine the source and effects of human activities which lead to pressures, by monitoring and assessing the status. While not detailing all methods, the aim of this overview has been to show tools which: (i) are fit for purpose; (ii) can cover the relevant temporal and spatial scales; (iii) have encompassed the range of marine responses to human activities and pressures, and (iv) have been tested with available data. In particular they have given assessments which are an integral part of making decisions and taking the necessary actions to ensure and/or improve that health. The assessment methods reviewed in this study share some common attributes, discussed below (see also **Table 1**), that provide lessons about key attributes needed for assessment of environmental status of open and coastal systems.

Assessments Should Use the Ecosystem Approach

All methods presented here are designed around the Ecosystem Approach. In the case of European methods, the MSFD requires that the member states that share the same marine region (i.e., Baltic, Atlantic, Mediterranean, and Black Sea) should collaborate to develop marine strategies in order to ensure coherence in the assessment, setting environmental targets and monitoring programmes. The regional platforms for developing coherent marine strategies are the Regional Sea Conventions (RSCs), which are the required regional coordination structures. Similarly, the MSFD states that "Marine strategies shall apply an ecosystem-based approach to the management of human activities," but no clear definition of the Ecosystem Approach is provided in the MSFD, although it is described elsewhere (e.g., CBD, 2000). The KnowSeas project definition (Farmer et al., 2012) provides a simple definition as: "a resource planning and management approach that recognizes the connections between land, air and water and all living things, including people, their activities and institutions." However, this definition does not specify how and by which means the Ecosystem Approach will be applied and what targets will be used. Those targets are dependent on each specific case that may vary among sea areas.

Therefore, using the Ecosystem Approach requires a common and explicit vision of the desired status of the environment, and multiple stakeholders need to be involved in the definition of that status. Within Europe, all RSC have stated their visions of the marine environment (**Table 3**) which emphasize the protection of ecosystem health and biodiversity as well as the sustainable use of marine ecosystem resources, which are implicit in the definition of GES of the MSFD. The next step is to decide upon strategic goals for fulfilling different aspects of the vision (e.g., health, diversity, and sustainability aspects; **Table 3**), and operational objectives for the different goals (Backer and Leppänen, 2008). Those objectives can be both science-based, evolving from the ecosystem state evaluations, or society-based describing potential threats impacting ecosystems (Laffoley et al., 2004).

Assessments Should Include Multiple Components of the Ecosystem

When applying an Ecosystem Approach in assessing environmental status, it is especially important to include both biotic and abiotic components of the natural system and a range of social components from the human system. The biotic components should be included in the assessment at different organizational levels (e.g., species, communities, biotopes) even though the assessments of the different levels may serve different purposes. For example, while information at the population level is required for stock evaluation, information at the community level is required for a broader biodiversity assessment. Similarly, as shown here, assessing community and ecosystem structure is central to surveillance monitoring, techniques for determining the cellular and individual health may be of more benefit in investigative or diagnostic monitoring (Elliott, 2011). The latter may also give early warning of change whereby deterioration in the health of a cell or individual, unless checked, will ultimately affect the population, community and ecosystem health (Tett et al., 2013). In turn, cellular (genomic) assessments as shown here may be of value in both explaining a likely response but also in predicting future changes to organisms and hence to populations and communities. Hence, the ecosystem level is represented by the combination of all species, habitats, communities, and their interactions, and the methods in this overview aim to include all these components.

In addition to the natural system, social components being monitored should include the many different ways that people interact with and benefit from natural systems. Of course, there are many potential indicators that can be used in the assessment of the components. In the case of the European MSFD, some of the 56 candidate indicators could potentially fulfill some of the desired criteria to be used and, at the same time, consider the characteristics, pressures, and impacts that are described in this directive (Teixeira et al., 2014).

Assessments Should Use Reference Conditions or Baselines and Be Repeated to Track Changes

The importance of setting targets and reference conditions in assessing marine ecosystem quality has been highlighted several times (i.e., Mangialajo et al., 2007; Gray and Elliott, 2009; Borja et al., 2012; Andersen et al., 2014). It is especially important to track the changes in marine status due to management measures being taken to reduce human pressures. Hence, it is necessary to repeat assessments both to inform new management objectives and to detect whether existing policies are effective, by measuring the discrepancy between the values of the monitored indicators and the reference conditions or target values set; this has been defined as true monitoring as opposed to surveillance (Gray and Elliott, 2009). It is axiomatic that all environmental legislation aimed at preventing adverse effects due to human actions requires the current system to be assessed against what

TABLE 3 | Comparison of the visions of the Good Environmental Status (GES) characterized by the regional sea conventions, OSPAR (The Convention for the Protection of the Marine Environment in the North-East Atlantic), HELCOM (The Convention on the Protection of the Marine Environment in the Baltic Sea Area—the Helsinki Convention), UNEP/MAP (The Convention for the Protection of Marine Environment and the Coastal Region of the Mediterranean—the Barcelona Convention, implemented in the framework of UNEP/MAP), BSC (The Convention for the Protection of the Black Sea—the Bucharest Convention, implemented by the Black Sea Commission), and the Marine Strategy Framework Directive (MSFD).

OSPAR	HELCOM	UNEP/MAP	BSC	MSFD
N.E. Atlantic	**Baltic Sea**	**Mediterranean**	**Black Sea**	**All regional seas**
Clean, **healthy** and biologically **diverse** North-East Atlantic ocean, used **Sustainably**.	**Healthy** Baltic Sea environment, with **diverse** biological components functioning in balance, resulting in a good environmental/ecological status and supporting a wide range of **sustainable** human economic and social activities.	The **healthy** Mediterranean with marine and coastal ecosystems that are productive and biologically **diverse** for the **benefit** of present and future generations.	Preserve its ecosystem as a **valuable natural endowment** of the region, whilst ensuring the **protection of its marine and coastal living resources** as a condition for **sustainable** development of the Black Sea coastal states, well-being, **health** and security of their population.	***Good environmental status***' means that marine waters provide ecologically **diverse and dynamic** oceans and seas which are **clean, healthy and productive** within their **intrinsic conditions**, and the **use of the marine environment is at a level that is sustainable**, thus safeguarding the potential for uses and activities by current and future generations.

is expected in an area if the actions were not present. For example, EIA, the WFD and MSFD, in Europe, and the Clean Water and Oceans Acts, in the US, all rely on detecting change from a known baseline, target, threshold, or reference value or determining a trend against the preferred situation (Borja et al., 2008). All of the methods reviewed here rely on the use of reference conditions to assess and track changes in the status; in turn this requires methods and calculations that can be repeated to enable future assessments with new information to be comparable. Repeatability is thus one fundamental characteristic of an ideal assessment.

Use an Integrative Assessment of All Components

We emphasize that by definition an integrative assessment must include multiple ecosystem components (e.g., biological, chemical, physical, social, economic), numerous biodiversity elements (e.g., from microbes to cetaceans), different assessment scales (e.g., from local, to regional and global sea scale), some criteria to define spatial scales and some guidance on integrating information (see a review in Borja et al., 2014).

Once the indicators, each with their specific targets or reference conditions, have been set, tested, and validated and the monitoring programmes implemented to provide data for those indicators, the assessment cycle can be completed (e.g., for MSFD; **Figure 9**). Thematic, holistic assessments need to integrate indicators addressing different aspects of the ecosystem, as shown by all the methods described here, to indicate the overall ecosystem level health of the marine region as well as the spatially expressed pressure and impact indices (Korpinen et al., 2012).

Some authors (Borja et al., 2014) have concluded that any integration and aggregation principle used should be ecologically relevant, transparent and well documented, to make it comparable across different geographic regions, as exemplified by the methods reviewed here although they do differ in the way in which this is achieved. Some of the methods rely on an overall thematic integration, for example, the HELCOM HOLAS tool uses the themes biology, chemistry, and supporting indicators.

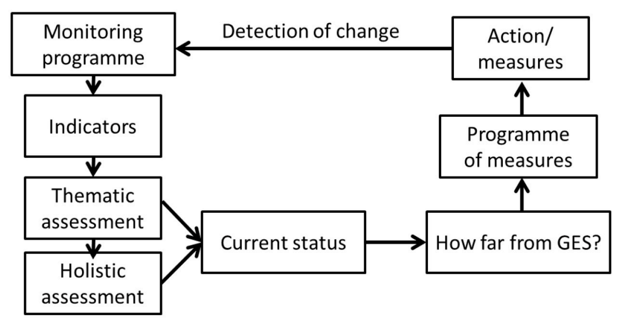

FIGURE 9 | The overall cycle for the assessment of the marine ecosystem status that links marine monitoring, indicators, thematic assessments, holistic assessment, and programme of measures in order to detect changes in the status of the marine ecosystems and to assess how far the current status is from the Marine Strategy Framework Directive's Good Environmental Status (GES).

The method from the Bay of Biscay groups indicators into four interlinked systems of ecosystem components. Another way of integration is to follow some external scheme such as the MSFD descriptors and subsequent criteria, as implemented in the MARMONI tool. Used in an unreflective manner, this can, however, involve some difficulties such as double counting the same ecosystem feature under different criteria (Berg et al., 2015).

Furthermore, there is the continuing discussion regarding whether an assessment of status should be a single value into which is embedded many descriptors or indicators or whether each element should be presented with its own quantified status. For example, in Europe, there is a continuing debate regarding whether the environmental status is presented as one single outcome (pass or fail), for a sea area by merging the assessments of all Descriptors, or whether each descriptor should be assessed independently and so a sea area would have 11 (one per Descriptor) indications of pass or fail at environmental status. The former approach has the benefit of simplicity in communicating the results (i.e., a sea area can be regarded as having passed or failed a definition of environmental status) whereas presenting 11 separate indications of the status allows a cause of failure to be readily identified (if, for example an area failed the Descriptor for seafood contamination but passed the other descriptors then management actions are more identifiable).

Use a Range of Values for Capturing Status

A value for the "deviance from target" is needed for planning the programme of measures and management actions to reduce or remove human pressures by controlling societal activities and drivers. This means that the assessment methods should show the variation in the status value. Usually this can be done through continuous ranges between 0 (bad status) and 1 (high status), as in the case of the methods for the WFD (see Birk et al., 2013). It has been adopted also for several of the methods reviewed in this study, for example the OHI (Halpern et al., 2012, 2015b; in this case uses a range from 0 to 100). The only method which has no continuous range is MARMONI, employing the binary scheme of only 0 and 100 as distinct values.

The MSFD similarly and implicitly uses a binary scale as it classifies an area as either in or not in GES. Using a common scale has the advantage of making assessment methods comparable, through intercalibration exercises, as those organized in Europe for the WFD implementation (Birk et al., 2013). Surprisingly, and in contrast to the WFD, the MSFD does not explicitly require intercalibration but the inescapable conclusion from the analysis here is that any member States, region or sea area using different methods will require intercalibration to demonstrate the coherence in application and implementation.

Weighting Components When Integrating

Sometimes, weighting indicators when combining them allows comprehensive assessments to recognize and capture that some information is more relevant or directly related than other information. All tools reviewed here have a weighting option, allowing managers to give more weight to indicators or features taking into account: (i) the spatial and temporal variability of the indicator; (ii) the availability of reliable data; (iii) the accuracy of assessing methodologies for each indicator, and (iv) the differential response of each indicator to the main pressures in the area, among others. NEAT is the only method in this review not applying the weighting to the indicators but rather use ecosystem features for weighting. Thus, the weight (influence) of an indicator on the assessment result is determined by, for example, the size and/or quality of an area to which the indicator is applied (Probst and Lynam, 2016). This allows giving due weight to the major ecosystem components, which are much easier to characterize than the major indicators, although this depends on how the weight system is defined (Probst and Lynam, 2016).

As highlighted by Borja et al. (2014), an adequate basis for assigning weights is not always available and in such cases equal weighting is recommended by Ojaveer and Eero (2011). However, assigning weights often involves expert judgment and some degree of subjectivity, and Aubry and Elliott (2006) point out that in some cases, expert opinions on weights can show important divergence even though best expert judgment may be the most defendable and acceptable method.

Calculate the Uncertainty Associated with the Assessment

Management of human activities to ensure GES naturally requires a solid foundation and a defensible approach, before decisions are made that may potentially have large economic consequences. Hence, it is important to ensure high confidence in the marine status assessment. Confidence quantification of the integrated status assessments has so far generally been neglected due to the complexity of such calculations. Only NEAT investigates the propagation of uncertainties from inputs of indicator values to the overall assessment in a quantitative way (using the Monte-Carlo method as described above), whilst the other methods assess uncertainty in a qualitative way. However, it is essential to associate indicator values with an uncertainty estimate which can be quantitative (as in case of natural variability) or qualitative (as in case of conceptual uncertainties behind the indicator). Unfortunately, most studies developing marine indicators do not consider indicator uncertainty or do not indicate how to calculate the uncertainty. The indicator uncertainty can be calculated based on estimates of various uncertainty components affecting observations used for the indicator, and the number of observations required to achieve a given accuracy and precision can be calculated (Carstensen, 2007). It is paramount that more focus is devoted toward quantifying the uncertainty of indicator values and how these affect the overall integrated assessment. Without knowing the confidence in marine environmental status assessments, or if the uncertainty is too large, decision-makers may decide not to adopt any measures to regulate human activities, due to the lack of precise information, especially if such measures have a high cost and uncertain outcome.

Ensure Comparability across Regions and Time

All of the methods reviewed here allow spatial and temporal comparisons within and between regional seas but each have strengths and weaknesses which need to be considered to improve the assessments and their confidence in managing marine ecosystems. Give that the type of assessments described here are enshrined in marine governance (Boyes and Elliott, 2014), such as the European MSFD and the US Oceans Act, and in licensing or marine activities (such as national pollution control legislation) then again it is emphasized that it is increasingly possible that there will be legal challenges to the science being used. Hence, the methods have to be robust and legally defendable both inside and between countries and at one time and across various reporting periods (e.g., Hering et al., 2010).

Use of Robust Monitoring Approaches and Data

As shown in the section Need of Innovative and Cost-Effective Monitoring, the monitoring methods are evolving and improving and thus the assessment methods or frameworks need to be sufficiently flexible to incorporate data acquired using new studies, instruments and methods, and which are used to derive new indicators with their own targets. The methods presented here can receive data from multiple sources and monitoring networks, making them sufficiently flexible to incorporate new indicators, for an Ecosystem Approach assessment.

Approaches Should Address Pressures and Impacts

Elliott (2014) showed the need for a holistic marine management, which is focussed around a risk assessment and risk management approach, which accounts for vertical governance systems and horizontal integration across stakeholders. Successful and sustainable marine management relies on the detection of changes in pressures, state, and impacts on human welfare but then, following the implementation of responses and measures, it addresses the drivers and activities in the marine arena and the catchments affecting it. Reducing human impacts on marine ecosystems, produced by pressures, requires a scientific basis for any management measures and ultimately the need for spatial predictions of environmental status (Andersen et al., 2015). An independent verification of the cause of the problem requires pressure indicators especially as the presence of an activity cannot be assumed to cause a pressure. For example, seabed extraction of sand does not have to cause smothering if mitigation measures are employed. However, those pressure indicators have to accommodate the fact that the pressure impacts have different spatial and temporal scales depending on the activity footprints, the pressure types and trajectories and the species they affect and therefore the pressure-state link may not always be within detectable timeframes. Including the timescales to the assessment tools is, nonetheless, within our reach.

CONCLUSIONS

Assessing the status of marine ecosystems under an Ecosystem Approach is fundamental to informing management decisions, and assessment frameworks have been developed to fit this need. As these frameworks are applied through time and to different regions, improvements with new information and increased understanding will be incorporated. Characteristics that are paramount to marine assessment frameworks include (i) transparency in describing which decisions were made and why; (ii) being scientifically defendable by being based on a sound conceptual understanding; (iii) repeatability, so change can be tracked through time, through understanding and quantification of uncertainty via access to detailed methods and computational code, and (vi) communicability of methods and scores through distillation and visualization to wide audiences (modified and expanded from Lowndes et al., 2015). Conducting assessments with these characteristics will not only make future assessments comparable between marine regions and through time for management interpretation but will also reduce the time and resources required for subsequent assessments and at the same time make the assessments legally defendable.

AUTHOR CONTRIBUTIONS

AB and ME conceived the paper. All authors have contributed in an equal manner to the preparation of the paper, the introduction and lessons learned. Then each author has had a part of the paper to write: ME, SK, and JA that of pressures, NR and AB that of monitoring, HOLAS method by JA, SK, and AH, Bay of Biscay by AB, OHI by BH and JL, MARMONI by GM, NEAT by JC, JA and CM.

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frontiers in
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METHODS ARTICLE
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Integrated assessment of marine biodiversity status using a prototype indicator-based assessment tool

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Integrated assessment of the status of marine biodiversity is and has been problematic compared to, for example, assessments of eutrophication and contamination status, mostly as a consequence of the fact that monitoring of marine habitats, communities and species is expensive, often collected at an incorrect spatial scale and/or poorly integrated with existing marine environmental monitoring efforts. The objective of this Method Paper is to introduce and describe a simple tool for integrated assessment of biodiversity status based on the HELCOM Biodiversity Assessment Tool (BEAT), where interim biodiversity indicators are grouped by themes: broad-scale habitats, communities, and species as well as supporting non-biodiversity indicators. Further, we report the application of an initial indicator-based assessment of biodiversity status of Danish marine waters where we have tentatively classified the biodiversity status of Danish marine waters. The biodiversity status was in no areas classified as "unaffected by human activities." In all the 22 assessment areas, the status was classified as either "moderately affected by human activities" or "significantly affected by human activities." Spatial variations in the biodiversity status were in general related to the eutrophication status as well as fishing pressure.

Keywords: biodiversity, marine, integrated assessment, habitats, communities, species, Marine Strategy Framework Directive

INTRODUCTION

Assessments of biological diversity have the ambitious objective of describing the state of an entire ecosystem, often by using only a few selected indicators. The challenge of this objective is to select a representative set of indicators, which fulfill the needs of science and marine policy. The EU Marine Strategy Framework Directive (MSFD) sets 11 qualitative descriptors for "good environmental status" (Anon, 2008), laying a common framework for all European marine biodiversity assessments. In this new assessment regime, biodiversity is considered to include not only the species diversity and the state of populations and habitats, but also seafloor integrity and food webs. Despite the detailed guidance on the selection of indicators (Anon, 2010), the MSFD does not provide a methodology to assess the overall state of marine ecosystems with the proposed criteria and indicators. Instead the EC tasked ICES with the production of detailed reports on the next steps of the implementation of the MSFD descriptors (see Cardoso et al., 2010 and relevant background reports).

Biodiversity assessments generally need to take into account the fact that marine biodiversity is sensitive to and also structured by natural factors such as salinity, currents, temperature, etc. More specifically, marine biodiversity assessments have been limited by the lack of integrated monitoring networks, high-quality biodiversity indicators, and indicator-based assessment tools (Borja, 2014), partly a consequence of the vast nature of biodiversity. We hypothesize that all three deficiencies are related to two shortcomings in monitoring. Firstly, monitoring of marine biodiversity is often expensive compared to the monitoring of eutrophication and contamination and good proxies for biodiversity changes have not been developed. Secondly, for certain features of marine biodiversity, e.g., seabirds, monitoring is inadequately integrated with the existing marine environmental monitoring and, hence, resources are wasted in uncoordinated efforts.

Consequently, assessments of marine biodiversity are not as well-developed as other types of assessments, where multi-metric indicator-based assessment tools are commonly used (HELCOM, 2010; Andersen et al., 2011). The regional sea conventions in the Baltic Sea (HELCOM; www.helcom.fi) and North-East Atlantic (OSPAR; www.ospar.org) as well as EU Directives (Habitats Directive and MSFD) call for assessments of biodiversity, but only HELCOM has thus far made an attempt to develop an prototype indicator-based tool for an assessment of biodiversity (HELCOM, 2009b, 2010).

A few recent studies of marine biodiversity in Northern Europe are based on data addressing a wide range of biodiversity features (such as phytoplankton, benthic communities, fish, seabirds, marine mammals) and robust and transparent scientific methods, e.g., Certain et al. (2011), Ojaveer et al. (2010), and Ojaveer and Eero (2011). These studies do not, however, take into account numerical biodiversity targets, and this is a shortcoming in regard to assessment of biodiversity status in the context of the MSFD (Anon, 2008).

In this study, we introduce and describe a simple indicator-based methodology (i.e., tool) for assessing the status of marine biodiversity. The tool is tested in Danish marine waters using provisional indicators with associated numerical target values and the results presented and discussed should accordingly be regarded as tentative. The assessment of biodiversity is made despite the lack of a commonly accepted definition of "marine biodiversity." Both the tool and the assessment are anchored in a Baltic Sea-wide conceptual understanding of "good biodiversity status" (HELCOM, 2010), where the overall vision is a healthy Baltic Sea with a favorable biodiversity status, including (1) natural marine and coastal landscapes, (2) thriving and balanced communities of plants and animals, and (3) viable populations of species. Hence, our understanding of "marine biodiversity" is broad and includes other elements than just a count of the number of species.

METHODS

We have developed a methodology for classification of "biodiversity status," employing a tool named Biodiversity Assessment Tool (BEAT) 2.0, which is an improved version of the HELCOM Biodiversity Status Assessment Tool (BEAT 1.0). This multimetric indicator-based tool was initially developed for integrated assessment of the status of biodiversity in the Baltic Sea (HELCOM, 2009a, 2010), but its updated version differs from its predecessor by having an improved fit with the EU MSFD descriptors, three status classes, a balanced approach to confidence rating as well as a more user-friendly appearance, where information about the Biodiversity Quality Objective (BQO) as well as interim (per category) and integrated classification results are presented.

BEAT 2.0 is an indicator-based assessment tool. For an individual indicator, synoptic information is required regarding reference conditions (RefCon), acceptable deviation from reference conditions (AcDev), and observations of the present state of biological diversity (Obs). AcDev is defined as a fraction or percentage of the RefCon, and is set site-specifically per indicator.

In calculating the status, we considered two types of indicators: (1) indicators that show a positive (+ve) response to human pressure factors, i.e., whose value increases with greater degradation in biodiversity (e.g., primary production, which is positively correlated to nutrient enrichment), and (2) indicators with a negative (−ve) response, i.e., whose value decreases with greater degradation (e.g., depth distribution of submerged aquatic vegetation, which is negatively correlated to nutrient enrichment or population size of a fish species, which is negatively correlated to fishing pressure).

As a first step, a BQO, which defines the border between "biodiversity status unaffected by human activities" (UN) and "biodiversity status moderately affected by human activities" (MO), is calculated per indicator:

$$\begin{aligned} \mathrm{BQO} &= \mathrm{RefCon} \times (1 + \mathrm{AcDev}) && (+\,\mathrm{ve\ response}) \\ &= \mathrm{RefCon} \times (1 - \mathrm{AcDev}) && (-\,\mathrm{ve\ response}) \end{aligned} \tag{1}$$

Step 2 is calculating the state value for each indicator through comparison with the BQO to determine indicator status. For example, for an indicator with +ve response, if the observed state (Obs) does not exceed the BQO, then the status "unaffected by human activities" is achieved. If the BQO is exceeded, the status is "moderately" (MO) or "significantly affected by human activities" (SI).

$$\begin{aligned} \mathrm{Status} &= \mathrm{UN} && (+\,\mathrm{ve\ response,\ Obs} \le \mathrm{BQO}) \\ &= \mathrm{MO/SI} && (+\,\mathrm{ve\ response,\ Obs} > \mathrm{BQO}) \\ &= \mathrm{UN} && (-\,\mathrm{ve\ response,\ Obs} \ge \mathrm{BQO}) \\ &= \mathrm{MO/SI} && (-\,\mathrm{ve\ response,\ Obs} < \mathrm{BQO}) \end{aligned} \tag{2}$$

Step 3 is to calculate a Biodiversity Quality Ratio (BQR), which in principle is comparable with the Ecological Quality Ratio principle *sensu* the WFD (Anon, 2000; Andersen et al., 2011). The BQR approach used in this assessment marks the ratio (0–1) between Obs and RefCon. For indicators with a positive response the BQR is given by RefCon/Obs. For those having a negative response the BQR is the inverse, i.e., Obs/RefCon.

$$\begin{aligned} \mathrm{BQR} &= \mathrm{RefCon/Obs} && (+\,\mathrm{ve\ response}) \\ &= \mathrm{Obs/RefCon} && (-\,\mathrm{ve\ response}) \end{aligned} \tag{3}$$

This step represents a transformation of indicator-specific information regarding the state of biodiversity to a numerical value, where the BQR values for different indicators can be compared and combined.

As a step 4, indicators are combined within four categories: (I) broad-scale habitats, (II) communities, (III) species, and (IV) supporting indicators. The classifications are based on a weighted average of the BQO and BQR values within each category. Weights are established by expert judgment and used to balance indicators among different biodiversity components or correlated indicators (e.g., several fish indicators are down-weighted against single indicators for seabirds or mammals). If not specified otherwise, the weighting is kept neutral by giving each of the indicators equal weights. On the basis of the BQR and AcDev values, each category is given a quantitative assessment according to the principles described above for a single indicator. Individual indicators have only two "classes," i.e., "unaffected" and "impaired/affected." There are three category classes from "unaffected," to "moderately affected" and "significantly affected" by human activities. Whilst the boundary between "unaffected by human activities" (UN) and "moderately affected by human activities" (MO) is a simple weighted average derived from the indicator-specific BQOs, the boundary between "moderately" and "significantly affected by human activities" (SI) is a value of two times the criteria-specific BQO.

At step 5, the results of the four categories are combined by applying the so-called "One out—All out" principle *sensu* the Precautionary Principle (MSFD Preamble, section 27; Anon, 2008) to the Categories I–IV. This implies that the category most sensitive to human activities, i.e., scoring lowest, defines the overall status of biodiversity within an assessment sector.

In addition to the above-described classification of biodiversity status, we estimate the confidence of the data and of the resulting classification by applying a simple scoring system (see Andersen et al., 2010). This system was initially developed for estimation of the confidence in eutrophication classifications but can be directly transferred and applied, when assessing biodiversity status. The approach, which scores the data on RefCon, AcDev and Obs gives equal weight to each of these three factors. In order to balance BQOs and Obs, we have modified the weighting of the factors with 25% to RefCon and AcDev and 50% to Status. The final confidence of the assessment can range between 100 and 0% and is according to Andersen et al. (2010) grouped in three classes: High (100–75%), Acceptable (75–50%), and Low (<50%). A description of the confidence rating method is available online as Supporting Material (Annex S3).

All calculations and subsequent classifications are made within a spreadsheet (see the Supplementary Material). Tracking calculations per indicator and also the integrations made per category and integration made in order to arrive at a final classification of biodiversity status is transparent and straightforward.

The BEAT 2.0 tool was tested and demonstrated using data from Danish marine waters, which are located in two distinct marine regions, the saline North Sea and the brackish Baltic Sea (**Figure 1**). Comprehensive descriptions of the study area and environmental status can be found in HELCOM (2010) and OSPAR (2010). The test was made on the basis of 22 assessment sectors in the Danish marine waters (**Figure 1**). The assessment sectors were larger in the offshore waters where spatial variation of the biodiversity indicators was considered smaller than in the coastal waters.

The data used for testing of BEAT 2.0 were compiled from various sources. Data on submerged aquatic vegetation as well as plankton (chlorophyll-a), benthic invertebrate communities, and nutrient concentrations originate from the Danish National Aquatic Monitoring and Assessment Programme (DNAMAP; see Conley et al., 2000; Carstensen et al., 2006; Dahl and Carstensen, 2008; Hansen, 2013). Data originates from three sources which are specific to the following areas: (1) offshore parts North Sea, Skagerrak and Kattegat (assessment sectors 1, 2, 4, 5), (2) offshore part of the Arkona Basin and Bornholm Basin, which are parts of the Baltic Sea (sectors 21 and 22), and (3) Danish coastal waters (sectors 3 and 6–20).

The indicators in regard to offshore fish, seabirds and marine mammals, which should be regarded as provisional, were developed specifically for this study and were also used for an interim assessment of biodiversity status in the North Sea (HARMONY project; unpublished data). Indicators used in previous assessments of the state of the North Sea (OSPAR, 2010) and Baltic Sea (HELCOM, 2010) were used for benthic and pelagic habitats and communities as well as supporting indicators. Detailed

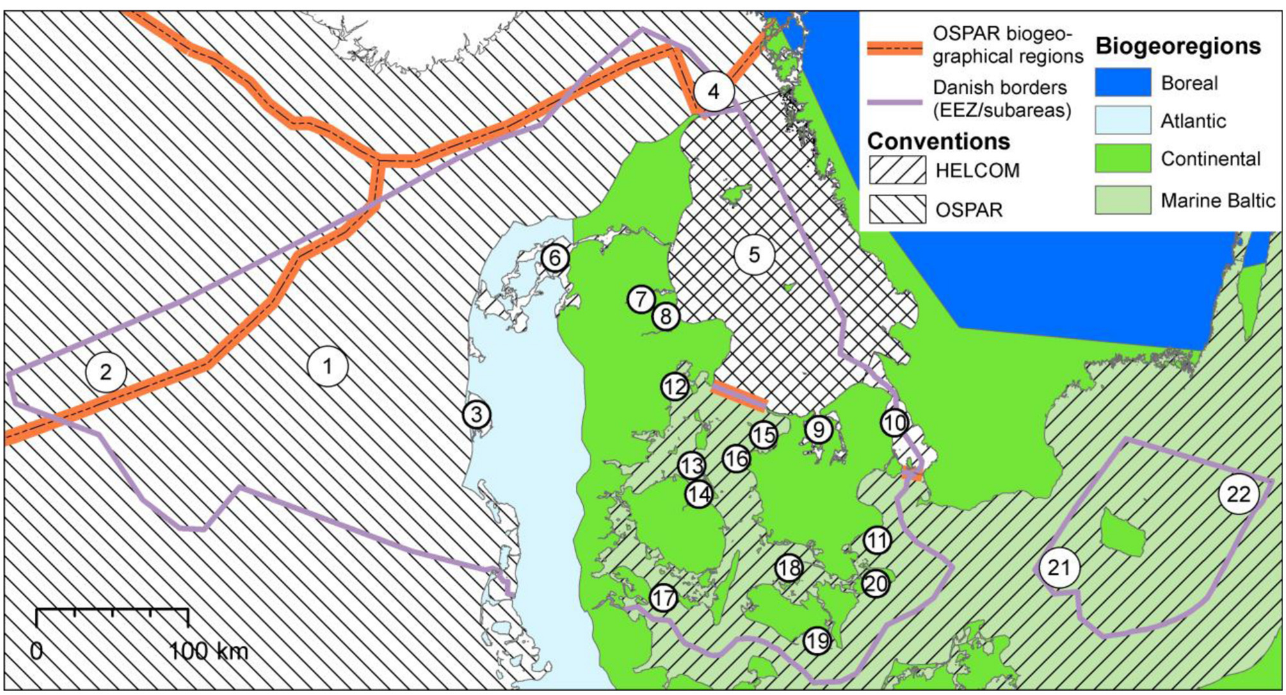

FIGURE 1 | Map of Danish marine waters. The borders indicated in the map represent the current MSFD boundary between the North Sea region and the Baltic Sea region, relevant OSPAR boundaries, relevant HELCOM boundaries as well as relevant Habitats Directive boundaries for biogeographical regions (BOR, Boreal; ATL, Atlantic; CON, Continental). Numbers indicates assessment sectors (see **Table 1** for names). Large circles indicate offshore assessment sectors, small circles coastal assessment sectors.

Table 1 | Assessment and classification of biodiversity status in Danish marine waters.

Assessment sector	Biodiversity Quality Ratio (BQR)				Integrated assessment
	C I	C II	C III	C IV	
1. NORTH SEA, eastern and southern parts	0.636*	0.907	0.656	–	SI
2. NORTH SEA, northern parts	0.700	0.904	0.619*	–	SI
3. Ringkøbing Fjord	–	0.377*	–	0.850	SI
4. SKAGERRAK, open parts	0.862	0.939	0.502*	–	SI
5. KATTEGAT, central parts	0.320*	0.749	0.482	0.733	SI
6. Limfjorden	–	0.351*	–	0.650	SI
7. Mariager Fjord	–	–	0.370*	0.519	SI
8. Randers Fjord	0.562	0.258*	0.485	0.369	SI
9. Isefjorden/Roskilde Fjord	–	0.613*	–	0.763	SI
10. The Sound, central parts	0.525*	0.823	–	0.560	MO
11. Fakse Bight/Stevns	0.843	0.704	–	0.336*	SI
12. Aarhus Bight	0.533*	0.671	–	0.548	MO
13. Marine waters north of Funen	0.353*	0.578	–	0.537	SI
14. Odense Fjord	0.294*	0.482	–	0.320	SI
15. Sejerø Bight	–	0.443*	–	–	SI
16. Kalundborg Fjord	–	0.357*	–	–	SI
17. Lillebælt, southern parts	0.230*	0.541	–	0.500	SI
18. Smålandsfarvandet	–	0.513*	–	–	SI
19. Rødsand	–	0.590*	–	–	SI
20. Hjelm Bight	0.838	0.702	–	0.533*	SI
21. ARKONA BASIN	0.534*	0.764	0.566	0.616	MO
22. BORNHOLM BASIN	0.553	0.239*	0.566	0.604	SI
Offshore assessment sectors (average)	0.601	0.750	0.565	0.651	–
Coastal assessment sectors (average)	0.522	0.534	0.428	0.540	–
All assessment sectors (average)	0.556	0.595	0.531	0.563	–

For each assessment sector, the weighted Biodiversity Quality Ratio (BQR) is presented. These values represent the perturbation in regard to the reference conditions. C I, marine landscapes (broad-scale marine habitats); C II, communities; C III, species; C IV, supporting indicators; MO, moderately affected by human activities; and SI, significantly affected by human activities. The category being decisive for the outcome of the integrated assessment and classification is marked with an asterisk. See Online Supporting material for details.

information about (1) the interim biodiversity indicators, (2) the sources for the monitoring data used as well as (3) the periods covered is available online as Supporting Material.

RESULTS

The average number of indicators per assessment sector was 10.2 ($n = 22$) ranging from 1 (no. 15 and 16) to 25 (no. 5). The average number of indicators in the four categories I–IV was 1.0, 4.0, 3.1, and 2.3, respectively. For the 6 offshore assessment sectors, the average number of indicators was 19.3 ranging from 8 (no. 22) to 25 (no. 2 and 5) and the average number of indicators in the four categories were 1.5, 5.8, 10.3, and 1.8 respectively. For the remaining 16 coastal assessment sectors, the average number of indicators was 6.8 ranging from 1 (no. 15 and 16) to 15 (no. 6) and the average number in the four categories were 0.9, 3.3, 0.3, and 2.4, respectively.

In the Danish marine waters, the average Biological Quality Ratio was 0.556, 0.595, 0.531, and 0.563 per category (**Table 1**). In category I, the BQR ranged from 0.230 to 0.862, in category II from 0.239 to 0.939, in category III from 0.370 to 0.656, and in category IV from 0.320 to 0.850.

For each assessment sector, a status classification was made per category and combined to a final integrated assessment of status per assessment sector (**Table 1**). The average of the lowest classified category was 0.433, ranging from 0.230 (sector 17: Southern Little Belt) to 0.639 (sector no. 1: North Sea, East+South). Areas with a BQR < 0.400 included Odense Fjord (sector 14), Little Belt (sector 17), and Bornholm Basin (sector 22), which all are significantly affected by eutrophication (HELCOM, 2010; Andersen et al., 2011). Areas with a BQR value above 0.600 were few and only found in the North Sea (sectors no. 1 and 2) and Isefjorden/Roskilde Fjord (sector 9). None of the assessment sectors were classified as unaffected by human activities. Three out of 22 assessment sectors were classified as moderately affected by human activities. The areas were Arkona Basin (no. 21), The Sound (no. 10) and Aarhus Bight (no. 12). The remaining 19 sectors were classified as significantly affected by human activities, and in 17 of these, the final classification was caused by categories I (broad-scale habitats), II (communities) or III (species). In two sectors, Hjelm Bight (no. 20) and Fakse Bight/Stevns (no. 11), the final classifications were a result of supporting indicators.

The confidence of the assessments was generally estimated to be above 50% and therefore considered acceptable (**Figure 2A**). However, two assessment sectors had a low confidence (no. 15 and 16: respectively, Sejerø Bay and Kalundborg Fjord) due to low number of indicators in the assessment in combination with challenges in regard to the setting of AcDev. Analysing the data per indicator revealed that monitoring data (State) and RefCon values on average had a higher confidence than the information on AcDev, which seemed to be slightly below the border between acceptable and low confidence (**Figure 2**). Scrutiny of the confidence per category revealed that all four categories on average had an acceptable confidence. All final classifications of the biodiversity status in the North Sea/Skagerrak area and the Kattegat had an acceptable confidence, while in the sub-division covering the Danish parts of the Baltic Sea, 2 out of 12 had an unacceptable confidence.

DISCUSSION

In this study we have presented a spreadsheet-based assessment tool for assessment of biodiversity, based on indicators, quantitative thresholds for good environmental status, and confidence rating. The assessment tool, tested by using both (i) existing and provisional indicators and (ii) recent data, showed that the marine biodiversity of Danish marine waters cannot be considered to be in good environmental status. The perturbations from reference conditions are indicative of human pressures in the assessment area (OSPAR, 2010; Korpinen et al., 2012).

Given the data and indicators available, we estimated the perturbations—understood as the deviation from reference conditions—represented by the lowest BQR values within an assessment sector. Parts of the North Sea and Skagerrak were less disturbed compared to the Kattegat and the Danish parts of the Baltic Sea (**Figure 3A**). The areas deviating most from reference conditions are all characterized by high nutrient inputs, high fishing pressure, and physical modification, sometimes caused by destructive fishing practices (HELCOM, 2010; Korpinen et al., 2012). Any measures to improve biodiversity status should as a priority address these key pressures.

An overview of the biodiversity status in the Danish marine waters revealed that a group of sectors being classified as moderately affected are interconnected (**Figure 3B**). The Sound is located downstream of Arkona Basin with a surface current from Arkona Basin to the west through Femernbelt between Denmark and Germany and to the north through the Sound. Hjelm Bight (sector no. 20) is located to the west and downstream of Arkona Basin. Fakse Bight/Stevns (sector no. 11) is located in between Arkona Basin and the Sound. The biodiversity status of the Arkona Basin and the Sound being classified as moderately affected by human activities is in line with the general understanding of the ecological status of these areas (HELCOM, 2010). Another sector having a slightly better status is Aarhus Bight (no. 12), where biodiversity status was classified as moderately affected by human activities in all the four categories. This, together with an estimated high confidence, does in our opinion confirm the classification. The reason for this slightly better status compared to adjacent sectors is most likely due to significant reductions in nutrient loads to Arhus Bight over past two decades (HELCOM, 2012).

Making an assessment without estimating the confidence of the result is a tendency, which in principle is unacceptable (**Figure 3C**). Estimating confidence is a statistical challenge, but the simple scoring system developed as a part of BEAT 2.0 overcomes this challenge in a non-statistical way and is able to cover confidence of threshold values, data and also the low number of indicators. This approach can be seen as temporary, leading to more sophisticated and data driven systems for assessment of confidence.

Many of the indicators in this assessment test have long traditions in previous assessments. Benthic communities and submerged aquatic vegetation have a long history in regard to assessments of eutrophication in the North Sea and Baltic Sea regions. Also indicators of fish communities have been used in previous assessments (Daan et al., 2005; Greenstreet et al., 2011), but reference levels had not yet been proposed for our study area, and for this analysis we used reference levels and acceptable deviations of 1 standard deviation based on the historic time series available.

Basin-wide biodiversity assessments have not hitherto included indicators for seabirds or marine mammals. The assessment in this respect can therefore be seen as a first attempt to use the trends in the population size of key species of seabirds or marine mammals as indicators of the status of the pelagic ecosystem in terms of habitat quality, food supply, and human-induced displacement. As the seabird data available for the assessment did not include data from the most recent period, the assessment used AcDev values of 50% and, hence, may give false positive impression of their status. Therefore, the reported changes in the abundance of fish-eating seabirds in the eastern parts of the North Sea, Skagerrak, and Kattegat should be regarded as strong indications of negative changes in the ecological status of these regions. Recent studies indicate that the regional reduction of fish-eating seabirds in the North Sea is mainly governed by changes in the large-scale abundance of herring (Fauchald et al., 2011). Reflecting the spatial caveats in the marine mammal data, the assessment used AcDev values of 50%. It is not known to what degree the impaired status of marine mammals in the eastern parts of the North Sea is a result of similar changes in the supply of pelagic fish which affected the abundance of seabirds in these regions. We did not include indicators for non-native species in this study. However, there is a growing understanding that, contrary to the normally negative perception of the ecological impact of non-native species, some species may provide significant ecosystem services in specific cases (Norkko et al., 2012).

In the current implementation process of the EU MSFD, there is a growing need to coordinate indicator development and agree on common sets of indicators, which allow coherent, trans-boundary assessments of the state of marine environment. By using existing indicators from the region, we noticed that several of the indicators were inherently correlated in nature (e.g., LFI and the slope of the size spectra, or chlorophyll a and Secchi depth) and using both as independent indicators in the present study may not be appropriate from a statistical point of view. In this study this correlation was accounted for by giving small weights to such indicators, but more stringent statistical

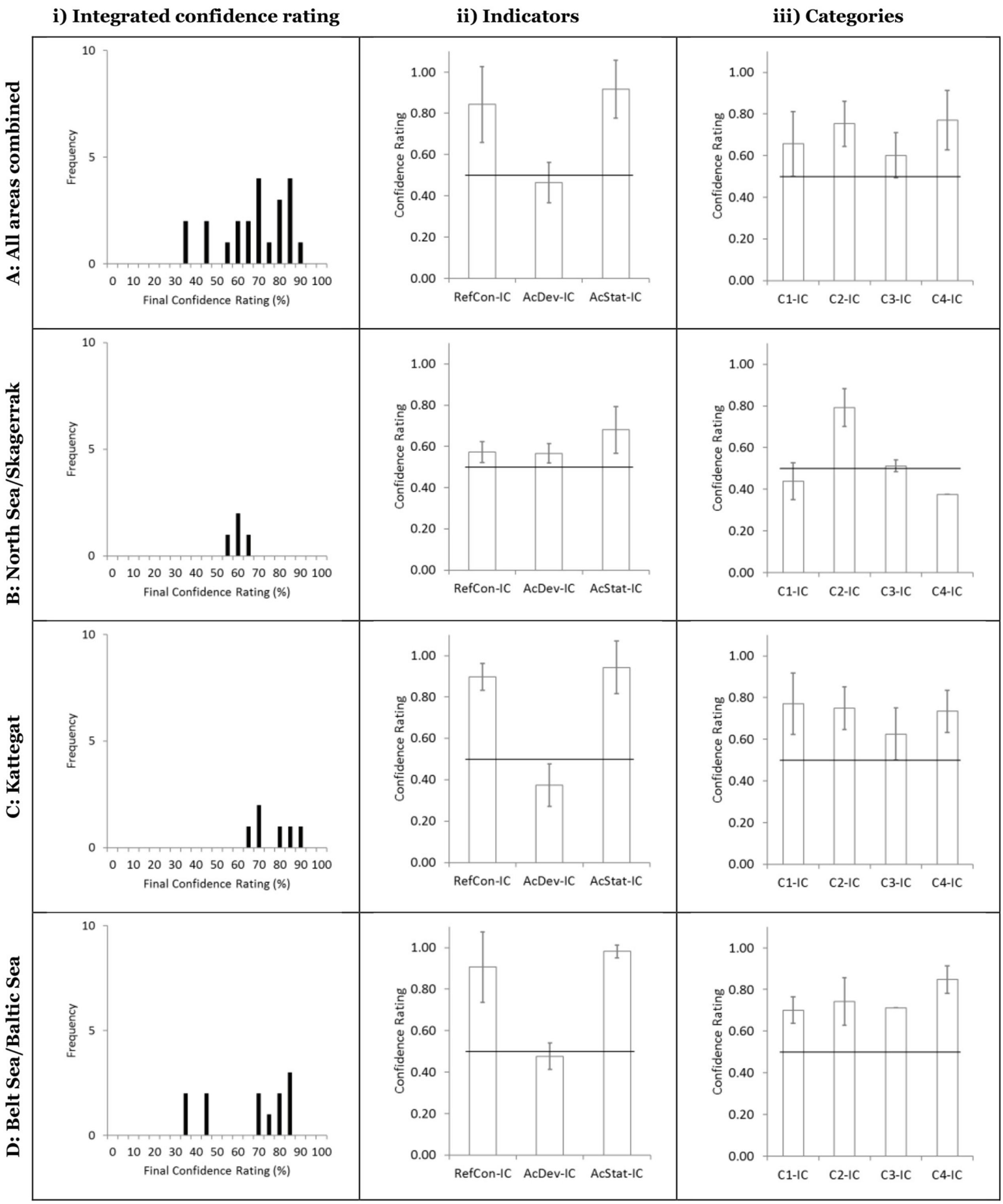

FIGURE 2 | (A) Confidence ratings made for (i) integrated assessments; (ii) information in regard to RefCon, AcDev, and AcStat of indicators, and (iii) categories I–IV. Values > 50% indicate an acceptable confidence (Andersen et al., 2010). **(B–D)** Sub-region-specific confidence assessments for the North Sea and Skagerrak, the Kattegat including the northern parts of the Sound and the Belt Sea and the western Baltic Sea. Please confer with Supplementary Material for details.

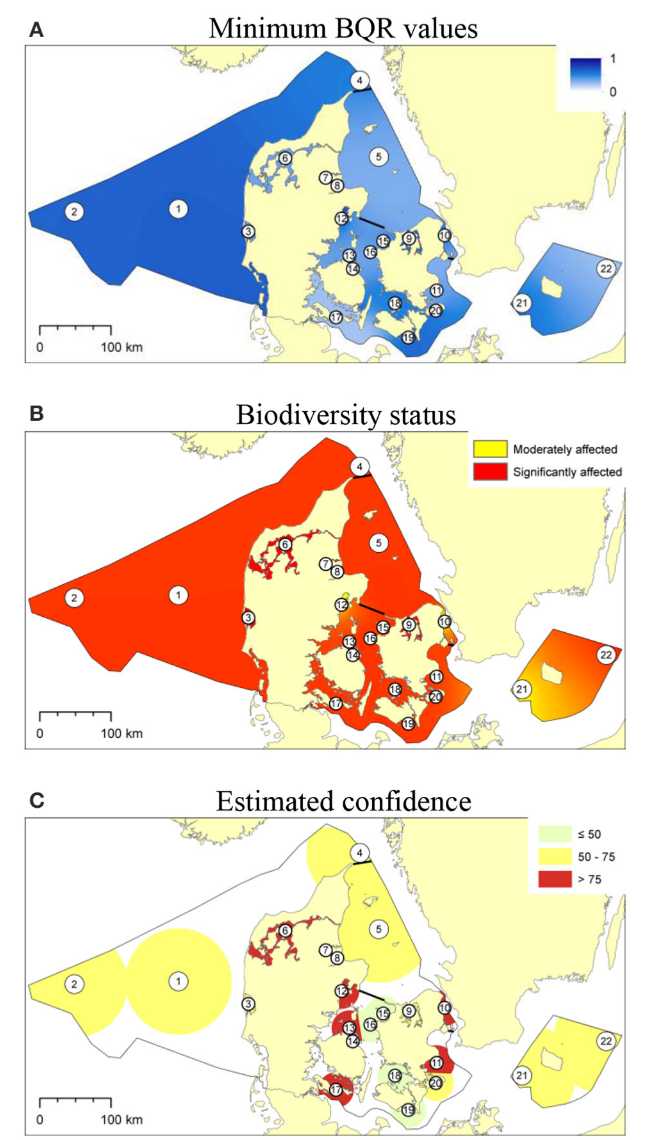

FIGURE 3 | Spatial distribution of BQR's (A), biodiversity status (B), and the estimated confidence (C). For illustration purposes representative points were chosen to represent the assessment sectors. From these points the results were spread to the marine area using a spline with barriers technique. For the six closed fjords (sectors 3, 6–9, 14) the values were spread evenly over the whole fjord.

consideration should be given to the issue before the next regional MSFD assessments.

We used supporting indicators to reflect changes in water quality in the Danish waters, which are affected by eutrophication (Ærtebjerg et al., 2003; Andersen et al., 2011). The eutrophication indicators indirectly reflect the condition of pelagic and benthic habitats and can, thus, indicate an overall status for a range of species and communities. Significant relations have been identified between nutrient loads and concentrations, chlorophyll-a concentrations, Secchi depth, depth limit of eelgrass (*Zostera marina*), total cover of macroalgae, and oxygen concentration in bottom waters (Conley et al., 2000; Nielsen et al., 2002a,b; Carstensen et al., 2004; Dahl and Carstensen, 2008). Thus, the water quality indicators can in a sense be called "true" indicators, as they can predict biological changes with simple methodology and relatively low costs. Nonetheless, in this study we considered them as "indirect" and prefer more direct measurements of biological parameters.

CONCLUDING REMARKS

Biological diversity in the Danish marine waters is significantly affected by human activities in most areas, but in a few sectors only moderately. None of the assessed sectors were classified as having a biodiversity status unaffected by human activities. The confidence of the assessments was estimated indirectly and generally regarded as acceptable, in a few cases even high. In two out of 22 sectors, the confidence was low indicating that monitoring of biodiversity in these sectors should be improved. The majority of the indicators were considered scientifically robust, but some indicators could, however, be further strengthened through production of peer reviewed scientific publications. Caution is also recommended in regard to the use of supporting indicators, especially in those few cases where they overrule biological indicators and thus determine the outcome of the integrated and final classification of biodiversity status. The BEAT 2.0 tool can support the EU Member States in the implementation of the MSFD, which specifically requires an overall assessment of the state of the marine environment as well as a specific assessment of biodiversity (Anon, 2008). The tool requires reliable indicators and quantitative thresholds for GES, but can function even with heterogeneous data availability. Assessments based on single indicators, though being simpler to link to human pressures, cannot reflect the variability and complexity of biodiversity responses required by the new assessments and therefore an integration of several indicators by an assessment tool is a prerequisite for the successful interface of science and environmental policy.

Finally, we would prudently like to remind the reader that there is no such thing as a perfect assessment tool. We do not promote the BEAT tool as such. We rather see this tool as a step for further development leading to better ecosystem-based tools for assessment, classification and adaptive management of marine biodiversity and human activities affecting marine life. The key challenges in regard to future integrated assessments of biodiversity status in marine waters are: (1) development of a wider range of biodiversity indicators representing different ecosystem components/food web categories, as well as (2) development of data driven methods for indicator integration and estimation of uncertainties.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fmars.2014.00055/abstract

Additional supplementary material, i.e., Annex S1 containing detailed information on data sources and provisional indicators used for the testing of the tool presented in this study, Annex S2 a summary of the confidence rating methodology, Annex S3 containing 22 individual BEAT classifications, and Annex S4 being a step-wise BEAT 2.0 tutorial, is available to the online version of this article.

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Indicator-Based Assessment of Marine Biological Diversity–Lessons from 10 Case Studies across the European Seas

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The Marine Strategy Framework Directive requires the environmental status of European marine waters to be assessed using biodiversity as 1 out of 11 descriptors, but the complexity of marine biodiversity and its large span across latitudinal and salinity gradients have been a challenge to the scientific community aiming to produce approaches for integrating information from a broad range of indicators. The Nested Environmental status Assessment Tool (NEAT), developed for the integrated assessment of the status of marine waters, was applied to 10 marine ecosystems to test its applicability and compare biodiversity assessments across the four European regional seas. We evaluate the assessment results as well as the assessment designs of the 10 cases, and how the assessment design, particularly the choices made regarding the area and indicator selection, affected the results. The results show that only 2 out of the 10 case study areas show more than 50% probability of being in good status in respect of biodiversity. No strong pattern among the ecosystem components across the case study areas could be detected, but marine mammals, birds, and benthic vegetation indicators tended to indicate poor status while zooplankton indicators indicated good status when included into the assessment. The analysis shows that the assessment design, including the selection of indicators, their target values, geographical resolution and habitats to be assessed, has potentially a high impact on the result, and the assessment structure needs to be understood in order to make an informed assessment. Moreover, recommendations are provided for the best practice of using NEAT for marine status assessments.

Keywords: biodiversity, assessment tool, MSFD, environmental status, spatial aggregation, integration, indicator sensitivity

INTRODUCTION

Biological diversity is widely recognized as one of the cornerstones of healthy ecosystems (e.g., Worm et al., 2006). Diversity may safeguard ecosystems against undesired regime shifts (Folke et al., 2004) and guarantee the continued delivery of ecosystem goods and services (Duarte, 2000; Beaumont et al., 2007). The need to maintain biodiversity is also recognized by international legislation (e.g., Convention of Biological Diversity; UNEP, 1992); to European Union (EU) level, the Marine Strategy Framework Directive (MSFD; European Union, 2008) requires its member states to assess the status of marine biodiversity and take action to guarantee that it remains at, or is restored to, Good Environmental Status (GES). A definition of what can be interpreted as good status can be consulted in Borja et al. (2013).

In order to conduct an assessment of status, and to determine the effectiveness of any implemented remedial measures, we need a clear definition of biodiversity and a unified approach for its assessment. In the marine assessments like MSFD, biodiversity is defined on the level of species, communities, habitats, and ecosystems, as well as in the genetic level (Cochrane et al., 2010). Indicators that show the ecosystem response to human pressures form the basis of the tool kit with which we can describe environmental status (Borja et al., 2016). Based on qualitative environmental objectives, targets are set for each indicator which allow policy makers to implement management measures should these not be reached (Borja et al., 2012).

One of the challenges faced during the first round of MSFD initial assessments is the diverging data availability for biodiversity across highly variable systems, but yet an overarching need to conduct compatible assessments across European regional seas (Hummel et al., 2015). European marine ecosystems comprise a complexity and variability both in space and time, ranging from fully saline systems such as in Mediterranean and Atlantic waters to the brackish Baltic Sea, and exposed open water systems such as in the northern Norwegian and Barents seas to fully enclosed systems such as the Black Sea. The levels of available knowledge and data within these systems vary, as well as the biological parameters and indicators used for assessments (Hummel et al., 2015).

The conclusions of the European Commission, in their evaluation of the EU member states' reports on the initial assessment carried out in 2010–2012 was that there is an apparent lack of coherence and comparability in the indicators used and in the final evaluation of the overall status, between the countries and within all regional seas (Palialexis et al., 2014). Therefore, there is an urgent need for coherent frameworks and methodologies to allow consistent approach in biodiversity status assessment across the European Regional Seas. This would also be needed in order to allow coherence in the biodiversity assessments for the EU Birds and Habitats directives and the EU Biodiversity Strategy 2020.

While we could argue that we cannot compare studies if we do not have directly comparable datasets, in practice this is rarely possible, and certainly not at large spatial scales, or involving multiple research institutes and member states. Since there is no single way of describing biodiversity that fits all purposes, and since regional seas have intrinsic differences, we need a pragmatic selection of indicators which are appropriate to the specific questions asked, as well as a flexible and transparent indicator-based tool for assessment of biodiversity status. There is a large number of operational indicators, which have been used to describe the status in different types of aquatic systems (Birk et al., 2012; Borja et al., 2016). As biological diversity is multifaceted, including different taxonomic and functional groups, it cannot be expressed with a single indicator. Consequently, sets of different indicators are needed to cover the broad aspects of biological diversity and it is their combination into a single assessment that becomes a challenge (Borja et al., 2014; Probst and Lynam, 2016). In order to obtain a single overall assessment value, or conclusion, the results of the multiple indicators used in the assessment need to be aggregated, depending on the purpose of the assessment; e.g., if the aim is to inform different stakeholders and to set overall targets for the improvement of the marine environment, or depending on the assessment scale (Borja et al., 2014). Clear and transparent aggregation and integration rules are needed to interpret indicator information onto an environmental status assessment (see Borja et al., 2014 for a review on integration methods).

A variety of assessment tools enabling the integration of indicators already exists (see e.g., HELCOM, 2009a; Andersen et al., 2014; Borja et al., 2016). However, only few of them have treated biological diversity in a comprehensive way, have been tested broadly (i.e., outside the region in which they have been developed), or consider the complexity at an adequate level of detail for the spatial scale for which they are applied. To overcome these issues, in the context of the EU funded project DEVOTES (DEVelopment Of innovative Tools for understanding marine biodiversity and assessing GES), the Nested Environmental status Assessment Tool (NEAT; Berg et al., 2016; Borja et al., 2016) has been developed to assess biodiversity status of marine waters under the MSFD. NEAT uses a combination of high-level integration of habitats and spatial units, and averaging approach (Borja et al., 2014), allowing for specification on structural and spatial levels, applicable to any geographical scale.

In this contribution NEAT is applied to the assessment of marine biological diversity in 10 different case studies distributed across the European regional seas (**Figure 1**). The assessment results are discussed, but the main focus of the paper is on: (i) analyzing the outcome of these assessments in light of the practical choices that have to be made to apply this tool, and (ii) proposing best practices for marine biological diversity assessment using this tool.

MATERIALS AND METHODS

Case Study Areas

The case study areas were selected to represent a wide range of marine systems (**Figure 1**), with different climatic and hydrographic characteristics as well as exposure to different human activities and management challenges (**Table 1**). These areas represent a wide range of marine biogeographical areas from subtropical waters to temperate and Arctic, covering the four European regional seas (i.e., Mediterranean, Atlantic, Black, and Baltic Seas). The surface areas of these case studies varied from <3000 km^2 in Saronikos Gulf (Greece) to >820,000 km^2 in the Barents Sea (Norway; **Table 1**). Detailed descriptions of the case study areas, with relevant references, can be found in Supplementary Material (S1–S10).

NEAT

NEAT is a structured, hierarchical tool for making marine status assessments (Berg et al., 2016; Borja et al., 2016), and freely available at www.devotes-project.eu/neat. In NEAT, the study area can be divided into hierarchical spatial assessment units

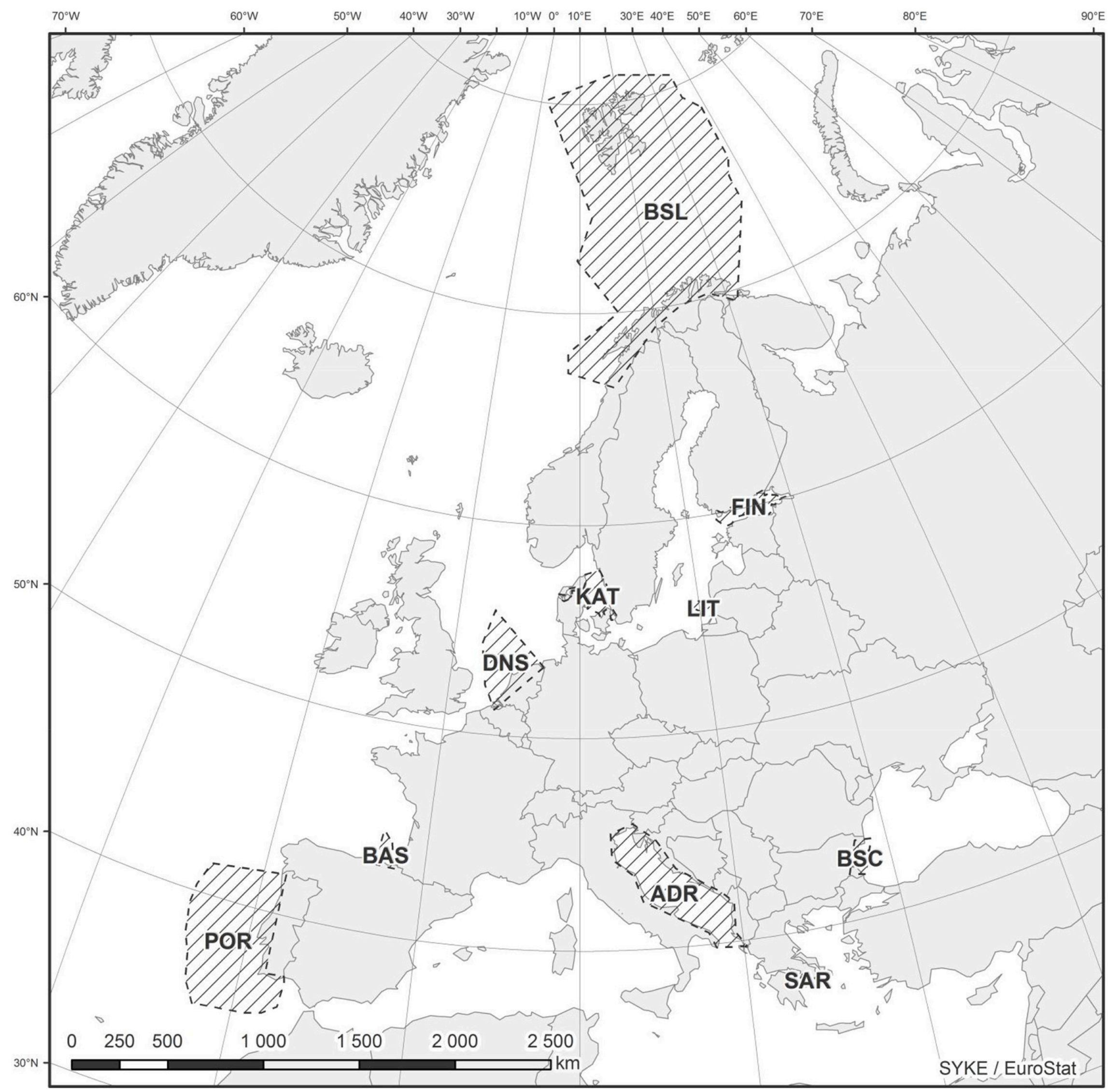

FIGURE 1 | The case study areas. For the area codes, see **Table 1**. More detailed case study maps can be found in Supplementary Material (S1–S10).

TABLE 1 | Characteristics of the case study areas.

Case study name	Code	Regional Sea, subregion	Salinity range	Seas Surface temperature range (°C)	Mean/max depth (m)	Special features	Main pressures	Biodiversity components in poorest status	Biodiversity components in best status	Observed gaps in biodiversity components	Observed gaps in habitats	Key references
Norwegian Barents Sea–Lofoten	BSL	North-East Atlantic, Barents Sea	34.4–35	−1.5–7.5, permanent/ seasonal ice cover/ice-free areas	230/500	Very large ecosystem; polar region	Climate change, fishing	Benthic vegetation, birds	Phytoplankton, Benthic fauna, zooplankton, fish	Lack of indicators for microbes, reptiles, large benthic organisms, cephalopods, alien species	Rocky and mixed substratum and biogenic reef (ex: Lophelia reefs)	(Wassmann et al., 2006)
Gulf of Finland	FIN	Baltic Sea	0–7	0–20, seasonal ice cover	37/100	Estuary; Heavy shipping traffic incl. oil tankers; Large drainage basin; Important resting and breeding area for migratory birds	Nutrient inputs, hazardous substances	Birds, water column habitat, benthic fauna, mammals	Zooplankton	Hard bottom fauna, microbes, alien species; habitat-level indicators	Hard bottoms	Ojaveer et al., 2010
Lithuanian marine waters	LIT	Baltic Sea	5–18	9.6–10.4	50/125	High wave exposure	Tourism, recreation, ports, shipping, urban, industry, agriculture, fisheries	Phytoplankton, birds, benthic vegetation	Benthic habitat, benthic fauna, benthic vegetation, zooplankton	Mammals		
Kattegat	KAT	Baltic Sea, North Sea	18–32 (surface) 25–35 (bottom)	2–19 (surface) 5–14 (bottom)	23.9/80	Transition zone between the Baltic Sea and the North Sea.	Nutrient inputs, hazardous substances, fishing, physical modification	Birds, phytoplankton	Zooplankton, fish	Non-commercial fish, alien species, microbes	Hard bottoms	Naturstyrelsen, 2012; Andersen et al., 2013

(Continued)

TABLE 1 | Continued

Case study name	Code	Regional Sea, subregion	Salinity range	Seas Surface temperature range (°C)	Mean/max depth (m)	Special features	Main pressures	Biodiversity components in poorest status	Biodiversity components in best status	Observed gaps in biodiversity components	Observed gaps in habitats	Key references
Dutch North Sea	DNS	North Sea	27.6–34.8	4–18	6/54	Shallow and highly productive area, soft bottom sediments, contrasted benthic habitats (stressed in the southern part, rarely disturbed in the northern one)	Beam-trawl fishing, organic loading, harbor infrastructures	Fish	Birds, mammals, benthic fauna	Benthic vegetation		Herman et al., 2014
Basque Coast	BAS	North-East Atlantic	32.5–35.5	11–23	Range 0–5000	High wave exposure, long fetch	Fishing/ shipping	Mammals	Phytoplankton, zooplankton, benthic habitat, water column habitat	Microbes	Deep and abyssal habitats	Borja and Collins, 2004
Portuguese continental subdivision	POR	North-East Atlantic	35–36	13–23	Range 0–6000	High wave exposure and seasonal upwelling at north-west coast, smoother conditions at south and south-west coast	Coastal trawling (also dredging, sediment disposal, harbors)	Fish, birds, pelagic fauna	Zooplankton	Reptiles, mammals, microbes. Species distribution and coverage area. The current status of all stocks has been assessed, although using secondary indicators	Deep and abyssal habitats and open-sea areas (>20 nm)	MAMAOT, 2012 (https://dl.dropboxusercontent.com/u/103729442/EstrategiaMarinha_subdv_Continente.pdf) Dupont et al., 2014
Black Sea coast (Large Varna Bay)	BSC	Black Sea	11–18	0–28	30	Hydrologically dynamic area	Tourism industry, urbanization, port activity, aquaculture	Fish, phytoplankton, zooplankton	Water column habitat, benthic habitat	Microbes, alien species, food-web indicators; insufficient fish monitoring	No monitoring in the open sea areas	National Initial Assessment GES Report, 2013

(Continued)

TABLE 1 | Continued

Case study name	Code	Regional Sea, subregion	Salinity range	Seas Surface temperature range (°C)	Mean/max depth (m)	Special features	Main pressures	Biodiversity components in poorest status	Biodiversity components in best status	Observed gaps in biodiversity components	Observed gaps in habitats	Key references
Saronikos Gulf	SAR	Mediterranean (Eastern Mediterranean/ Aegean Sea)	38–39	12–28	100/450	It is the natural marine gateway of the city of Athens and Piraeus harbor. It receives the effluents of the central sewage outfall of Athens through a deep underwater outlet.	Activities: Waste Water Treatment Plant (WWTP), Shipping, tourism, fishing. Pressures: input of organics/nutrients, contaminants, habitat loss, resource exploitation, alien species, litter	Mammals, fish	Reptiles	Birds, zooplankton		Simboura et al., 2014, 2015; SoHelME, 2005; SoHelFI, 2007
Adriatic Sea	ADR	Mediterranean	37–39	18–25	35/1200	Cyclonic circulation; input from the Po river	Organic loading; overexploitation; bottom trawling	Mammals, phytoplankton		Fish, birds, zooplankton	Reef and mud habitats	Artegiani et al., 1997a; Artegiani et al., 1997b; (UNEP(DEPI)/MED WG.408/Inf.14, 2015).

(SAU) and habitat types (HBT); e.g., SAU "archipelago zone" could include "inner archipelago" and "outer archipelago" as lower-level SAUs, and they, in turn, could include, e.g., water bodies as yet lower-level SAUs. Similarly, the HBT "seafloor" could include HBTs "soft bottom" and "hard bottom," which again could be further sub-divided (**Figure 2**). NEAT classifies the status of each SAU based on indicators that have been defined for that SAU; if one SAU has indicators describing different HBTs, the status of each HBT within a SAU is assessed first, and each HBT is then given equal weight in assessing the status of the SAU. The overall assessment is an average of the SAUs, weighted by their surface areas (km^2). Other weighting schemes can be applied, if desired.

Each indicator must be explicitly linked to a SAU and a HBT—the same indicator, e.g., "the maximum depth of seaweed," can be included multiple times for multiple SAUs and HBTs if it has been assessed for multiple areas. These instances of indicators are called "indicator values" in this paper, while the indicators describing a certain ecological concept, e.g., the growth depth of a macrophyte species, or the reproduction rate of a bird species, are called "unique indicators."

In order to aggregate indicators by weighted average, it is necessary to transform all indicators to a common scale. In NEAT, indicators are transformed into values that range from 0 to 1 using a continuous piecewise linear function. On this scale, the value of 0.6 corresponds to the boundary between good (>0.6) and not good (<0.6) status. Transformation to this scale is defined by specifying the values of the indicator in the original measurement scale, which corresponds to the transformed values of 0, 0.2, 0.4, 0.6, 0.8, and 1.0. Though the transformation function is piecewise linear, the definition of 5 segments allows a reasonable approximation to non-linear functions. These five segments are also used here for illustrative purposes, and they are called bad/poor/moderate/good/high classes, although it is recognized that the boundary between GES and non-GES lies between the "moderate" and "good" classes.

Indicator Selection and Specification

The indicators used for this assessment represent the best available data and expertise for the six biological descriptors of the MSFD [i.e., D1 (biodiversity), D2 (non-indigenous species), D3 (commercially important species), D4 (food webs), D5 (eutrophication), and D6 (sea floor integrity)] in each case study area. These indicators include the national and regional indicators used for the MSFD assessment, and indicators derived from scientific literature and expertise. They have been selected to be representative of various biodiversity components, habitats, and geographical areas relevant for each case study area; however it is possible that no indicators exist to be used for some relevant components. The list of indicators included in each case study is available in Supplementary Material S11.

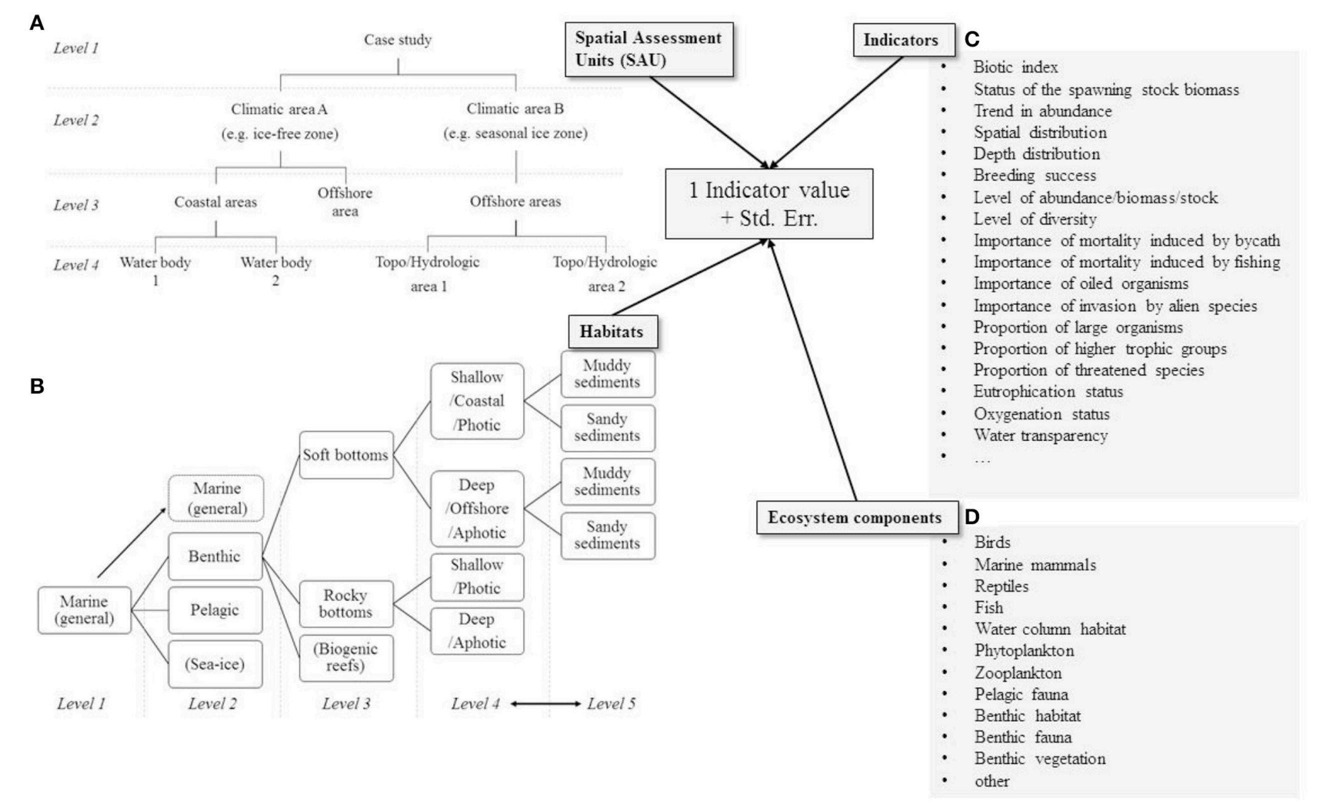

FIGURE 2 | The indicator values are specific for (A) a Spatial Assessment Unit (SAU) at any level of the hierarchy, (B) a habitat (HBT) at any level of the hierarchy, (C) an indicator which is definer for (D) an ecosystem component.

Each indicator is associated to an ecosystem component class that describes the ecosystem component that the indicator describes. In this study, 12 ecosystem components were defined in order to accommodate all indicators used in all of the case studies. These components were phytoplankton, zooplankton, fish, reptiles, marine mammals, birds, benthic fauna, benthic vegetation, pelagic fauna (composite indicators consisting of data from multiple pelagic fauna groups), all taxa (composite indicators consisting of data from multiple taxa), benthic habitat, and water column habitat. The latter two components gathered indicators related to physico-chemical conditions of the habitat, necessary to maintain life (e.g., oxygen or nutrients), whilst the "all taxa," benthic fauna, and pelagic fauna groups included composite indicators encompassing many species groups; the other nine ecosystem components were taxonomic groups.

Biodiversity Status

The status of the biological diversity was assessed for each case study area using NEAT. The analysis provides an overall assessment for each case study area and a separate assessment for each of the ecosystem components included in the assessment. The final value has an associated uncertainty value, which is the probability of being in a determinate class status (GES/non-GES). This uncertainty was determined by the standard error linked to the indicator values (Carstensen and Lindegarth, 2016).

Evaluation of Assessment Design and Its Effects on the Status Assessment

The application of NEAT to a broad range of marine regions provides an opportunity to test and compare the NEAT assessment approaches and evaluate the consequences of design choice for the general environmental status assessment. How the available data are combined within the tool might have consequences on the results of the status assessment of biodiversity (Borja et al., 2014; Probst and Lynam, 2016). Therefore, one of our aims is to evaluate the consequences of the way the assessment was designed on the general assessment result.

NEAT gives a framework to organize the assessment, but it does not prescribe the number of assessment components, i.e., indicators, SAUs, HBTs, or ecosystem components to be used in an assessment. The user has the option to organize the different components of NEAT depending on the case, e.g., the morphological characteristics of the area, availability and resolution of data, and how the selected local indicators are defined.

In order to describe the assessment design, the following key components were summarized for each case study: (i) the total number of SAUs and how many hierarchical SAU levels there are, (ii) the total number of HBTs and their hierarchical levels, (iii) the number of ecosystem components covered by the indicators, (iv) the number of unique indicators (i.e., not repetition of the same indicator on a different spatial unit), as well as (v) the quantity of data, defined as the number of different indicator values (e.g., if the same indicator is defined separately for five different SAUs, they would comprise five indicator values).

NEAT assigns weights to the indicators based on the SAU and HBT that they represent (see Section Evaluation of the Assessment Results). The SAUs are weighted according to their surface area and the HBTs are weighed equally within a SAU. Therefore, the indicator values contribute to the assessment with different weights, the highest weight being assigned to an indicator representing a large SAU with a small number of indicators, and within it a HBT with a small number of indicators. The relative weights of the indicator values were used to identify the indicators that contribute 90% of the weight of the final assessment. In addition, the relative weight of each ecosystem component in each case study assessment was calculated. These summary statistics highlighted differences in aggregating information among case studies.

To test the sensitivity of the case study assessments to the selection and number of indicator values, a sensitivity analysis was performed by running the assessment using randomly selected indicator values. The number of indicator values included into the assessment varied from 1 to the maximum number of indicators in the case study minus one. This process was repeated 100 times for each number of indicator values. For example, take a case study with 120 indicator values. First, one random indicator value is selected and the assessment is done using only that indicator. This procedure is repeated 100 times. Then, two indicator values are picked at random, and the assessment is run using them; this again is repeated 100 times. This procedure is repeated for all numbers of indicator values up to 119. This results in a large number of values whose divergence can be analyzed to see if any patterns can be identified.

RESULTS

Assessment Design

The number of SAUs as well as how many hierarchical levels were used in these varied widely between the case studies. The number of SAUs included in the Gulf of Finland and Portugal continental sub-division cases were much higher (>60) than in all other case studies which included, on average, 9 different SAUs. Excluding these two case studies, larger areas were usually assessed using more SAUs. The number of hierarchical SAU levels varied between 1 and 5, but in 7 out of 10 cases, there were 3 or 4 levels (**Table 2**, **Figure 2**). The total number of HBTs included in the assessment varied between 3 and 9, and 9 out of 10 case studies had 2 or 3 hierarchical HBT levels (**Table 2**).

Not all SAUs necessarily included all habitat types, and indicators or data may not exist for all defined HBT types for each SAU. The number of SAU-HBT combinations that were assessed by at least one indicator value, varied between 6 and 132 (**Table 2**).

The number of ecosystem components included in the analyses varied between 5 and 9, with an average of 7.3 (**Table 2**). It has to be noted that all ecosystem components identified in this study were not applicable to all areas; an example being reptiles that do not occur in most of the study sites.

The number of unique indicators applied in each case study area varied between 11 and 116 (**Table 2**, Supplementary Material S11). The number of indicator values varied greatly with 466

TABLE 2 | Synthesis of the structure used by the different case studies for the nested assessment.

Case study name	Area (km^2)	Number of SAU levels (total number of SAU)	Number of HBT levels (total number of HBT)	Number of SAU*HBT combinations with data	Number of ecosystem components included	Number of unique indicators (see Supplement 11)	Number of indicator values
Norwegian Barents Sea–Lofoten	821 478	4 (13)	3 (9)	21	7	40	74
Gulf of Finland	22 482	5 (60)	2 (3)	103	8	25	147
Lithuanian marine waters	6 426	2 (4)	2 (7)	6	9	27	50
Kattegat	17 440	3 (11)	2 (7)	21	8	31	69
Dutch North Sea	57 000	1 (1)	3 (6)	6	6	15	31
Basque Coast	10 794	3 (8)	3 (6)	22	9	48	109
Portuguese continental subdivision	268 645	4 (61)	4 (7)	132	7	14	466
Black Sea coast (Large Varna Bay)	1 434	3 (7)	2 (4)	15	7	35	112
Saronikos Gulf	2 907	3 (4)	3 (6)	10	7	17	29
Adriatic Sea	138 600	3 (10)	2 (5)	17	5	116	177
Mean	134 721	3.1 (17.9)	2.6 (6.0)	35.3	7.3	39.2	126.4
Stdev	255 807	1.1 (22.7)	0.7 (1.7)	44.3	1.3	30.7	128.8

SAU, spatial assessment unit; HBT, habitats. The case studies are ordered according to their latitude.

values at the higher end in Portugal continental sub-division and between 20 and 200 values in all other case studies (**Table 2**).

Biological Diversity Status

The summary of the test NEAT assessments of the 10 case study areas is presented in **Figure 3**. The assessment resulted in GES for the Basque EEZ and the Barents Sea-Lofoten, with 100 and 66% confidence, respectively, the remaining eight case studies presented non-GES (i.e., bad, poor, or moderate; **Figure 3**). Lithuanian coast has the potential for being in GES, but with a low confidence of 20% (**Figure 3**). For the other case studies, this probability of achieving GES was <1% (**Figure 3**).

The different ecosystem components showed different status in the case study areas (**Figure 4**). No strong pattern among the ecosystem components could be detected, but some commonalities were found: Indicators based on marine mammals generally indicated degraded situation in 6 cases out of 7 (**Figure 4**). When included, birds and benthic vegetation indicators as well as water column indicators of physico-chemical status also indicated degraded situation in 5 cases out of 7. Indicators encompassing several ecosystem components ("AT," on **Figure 4**) always indicated degraded situations. On the other hand, indicators of benthic habitats' physico-chemical status and of zooplankton community status indicated GES when they were included in the assessment (**Table 1**, **Figure 4**).

Relative Contribution of Indicator Values and Biodiversity Components

The indicator values contributed differently to the final assessment result (**Figure 5**); indicator values defined for larger SAUs tend to have more weight, particularly if there are only few indicators defined for these SAUs. In 7 out of the 10 case studies, <10 indicator values already contributed to more than 50% of the final assessment result. For 9 case studies, <50 indicator values contributed to >90% of the final assessment. This 90% of the final assessment was reached with <20 indicator values in five case studies (**Figure 5**). The five indicator values that made the highest contribution to the final assessments of each case study are listed in **Table 3**. These indicator values were dominated by mammal, bird, fish, and benthic fauna indicators.

The 12 different ecosystem components' contribution to the final assessment result did not correspond to the number of indicator values defined for each component (**Table 4**). For example, most case studies had a large proportion of benthic fauna indicator values (average: 22.4% of indicators values), which ultimately did not reflect proportionally in the final assessment (average contribution: 11.7%). In contrast, the proportion of fish and marine mammals indicator values were lower, but these components contributed to a higher proportion of the final assessment. In five case studies (i.e., Barents-Lofoten, Gulf of Finland, Dutch North Sea, Saronikos Gulf, and Adriatic Sea), "Benthic fauna" was the component with the highest proportion of indicator values (**Table 4**); the other five case studies each had a different component with the highest number of indicator values. However, in none of the case studies, benthic fauna was the component with highest contribution to the final assessment (**Table 4**); in five (i.e., Gulf of Finland, Dutch North Sea, Basque coast, Portuguese continental sub-division, and Black Sea coast) and two case studies (i.e., Barents Sea–Lofoten and Adriatic Sea) respectively, fish and mammals were the components carrying the highest weight to the final assessment (**Table 4**). However, other ecosystem components, that overall did not contribute to many case study assessments, were very relevant for specific case studies (e.g., the composite

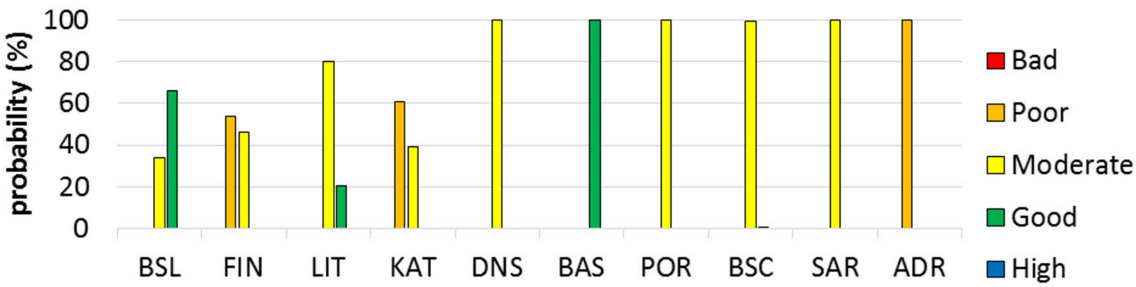

FIGURE 3 | Probabilities for the five environmental status classes for each of the 10 case study assessments. Good environmental status is assumed attained if the cumulative probability of "Good" and "High" is higher than the cumulative probability of "Moderate," "Poor," and "Bad." If opposite, the Good environmental status is not attained. For case study codes see **Table 1**.

group "all taxa" in the Saronikos Gulf and benthic habitat in the Lithuanian coast).

Sensitivity Analysis

The sensitivity analysis shows that there are major differences in how much the result varies if only a subset of the indicator values is included in the assessment (**Figure 6**). For example, if only a small number (close to 0) indicators were included, the assessment results in all studies could be anywhere between high and bad status, except in Barents Sea and Portuguese continental subdivision, where they could range from poor to high status. As more indicator values are added, the range of outcomes narrows down. However, how steeply that happens when indicator values are added varies between the case study areas (**Figure 6**).

DISCUSSION

The current NEAT-based assessment demonstrates a large-scale marine biodiversity assessment, providing a feasible solution to the apparent problem pointed out by the European Commission, in their evaluation of the EU member states' reports on the MSFD initial assessments carried out in 2010–2012 (Palialexis et al., 2014). This problem was the apparent lack of coherence and comparability in indicators used and in the final evaluation of the overall status between the countries and within all regional seas (Palialexis et al., 2014). Despite the available guidance and Commission Decision (European Union, 2010) on GES descriptors, criteria and indicators, the overall picture in assessments was patchy and non-coherent (European Commission, 2014). The use of NEAT, and its validation in different regional seas and case study areas, is a crucial contribution from the DEVOTES project to provide a harmonized approach and methodology for a coherent and comparable environmental status assessment across the European regional seas. It also shows that although the regional seas have different characteristics and human pressures impacting those (Claudet and Fraschetti, 2010; Micheli et al., 2013a; Andersen et al., 2015), a coherent assessment framework can be employed to evaluate differences in the environmental status and the ecological components that are impacted by different pressures.

The study and the comparison of the case studies brought into light several issues that need attention in order to improve the coherent and comparable "biodiversity status" assessments of the European regional seas. These issues are related to the data and indicator availability, how the assessments are structured, how the integrative assessment should be structured, and how this structure should be taken into account when defining the spatial resolution and indicator selection of the assessments. The current study revealed that while these assessments could be carried out, there are two major problems in achieving the objectives of the MSFD assessments: (i) there are still multiple gaps in the availability and coverage of indicators in the various areas, and (ii) comparability of the status assessments across different regions would benefit from a more unified assessment framework, even if indicators suitable for each area remained different. NEAT provides a general framework that could be accompanied with guidelines for the selection of SAUs, HBTs, and indicators.

Each of the case studies was initially designed with the best available selection of spatial units, habitats, and indicators, adhering to the NEAT methodology but without specific guidelines for the indicator selection, target level setting, etc. This situation resembles the situation where the new users would start using NEAT on their area. For the purposes of this study, the assessments were evaluated and harmonized to some degree, e.g., if the same indicator appeared in multiple case studies, it was ensured that it was associated to the same biodiversity component (e.g., chlorophyll a levels would be assigned to phytoplankton). Despite this harmonizing, there were major differences in how the case studies were constructed in terms of spatial resolution, habitats, and indicator definition. The current assessment is based on best available data and evaluation of the experts participating within this exercise, and the biodiversity status results of this study should be considered as indicative, not definitive.

The indicators selected for the assessments are designed or adapted for each area separately, including the geographical and habitat specification and the target level, i.e., which values are considered good and which less than good in any given area and habitat. This means that the "good" status is scaled according to the area: In areas with a naturally low biodiversity, lower

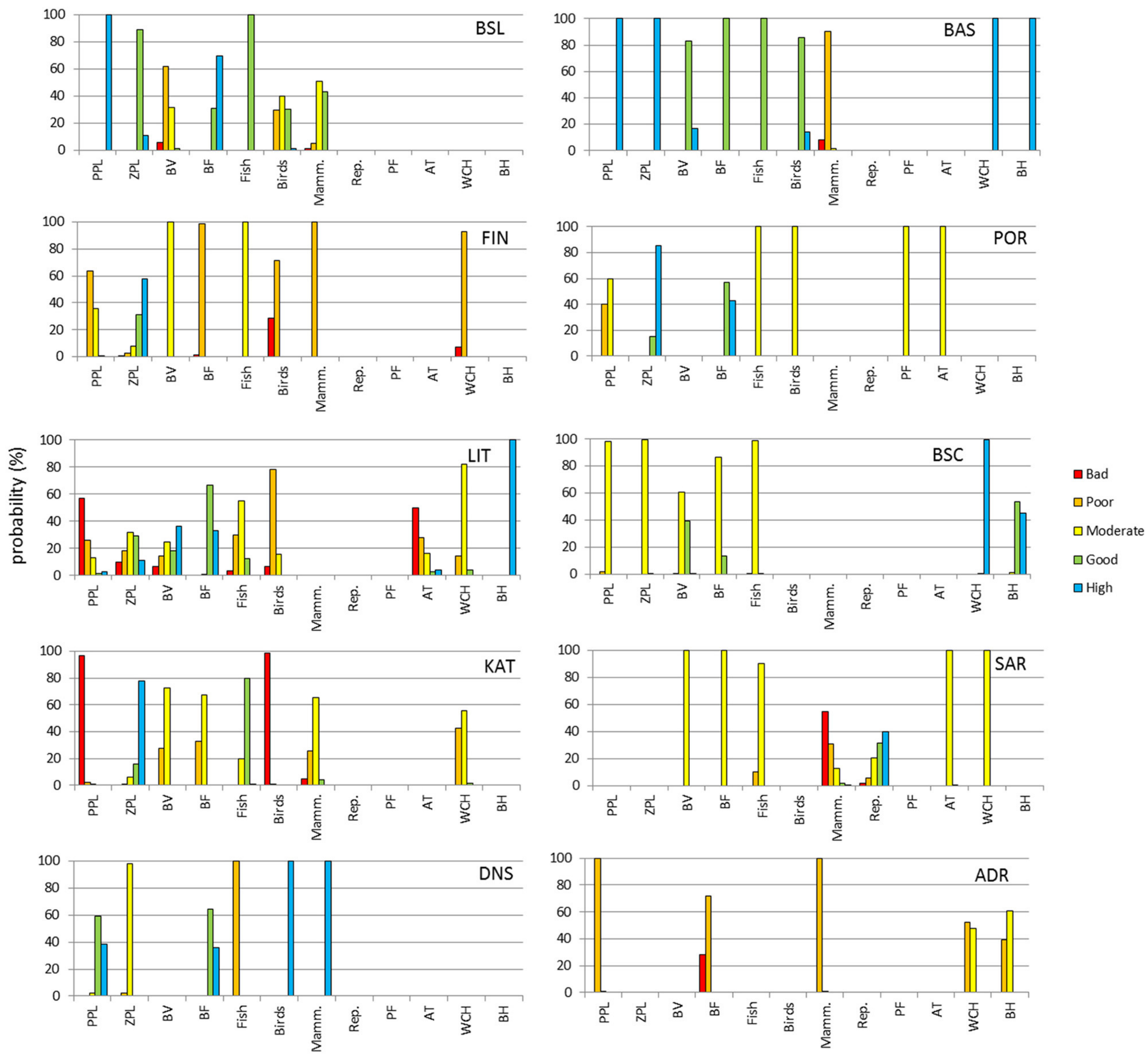

FIGURE 4 | Probabilities for the five environmental status classes separated by ecosystem component for each of the 10 case studies (for codes see Table 1). Ecosystem components considered are: Phytoplankton (PPL), zooplankton (ZPL), benthic vegetation (BV) benthic fauna (BF), fish, birds, mammals (Mamm.), reptiles (Rep.), pelagic fauna (PF), all taxa (AT), water column habitat (WCH), benthic habitat (BH).

biodiversity is also considered "good" than in areas with naturally high diversity. This makes the assessment relevant for each area, and the result must be interpreted to be in relation to undisturbed condition of that area rather than in absolute terms of diversity.

According to a categorization of rules or methods for combining or aggregating indicators or criteria within a given descriptor (Prins et al., 2013; Borja et al., 2014), NEAT is classified as a high-level integration method which reduces the risks associated to the "one out, all out" principle of the Water Framework Directive approach (Borja and Rodríguez, 2010) while giving an overall and specific (to descriptors and components) assessment.

According to the relevant guidance document for the MSFD (Prins et al., 2013), the spatial scales are not the same for all indicators within the biodiversity descriptor, where depending on the species or habitat a different spatial scale may be used. It is also recommended to address uncertainties and assess confidence of the classification result (as a secondary assessment). In our study, the NEAT software treats equally all assessment elements assigning equal weights, but gives more weight in cases of larger spatial coverage, with higher data representativeness, in that way

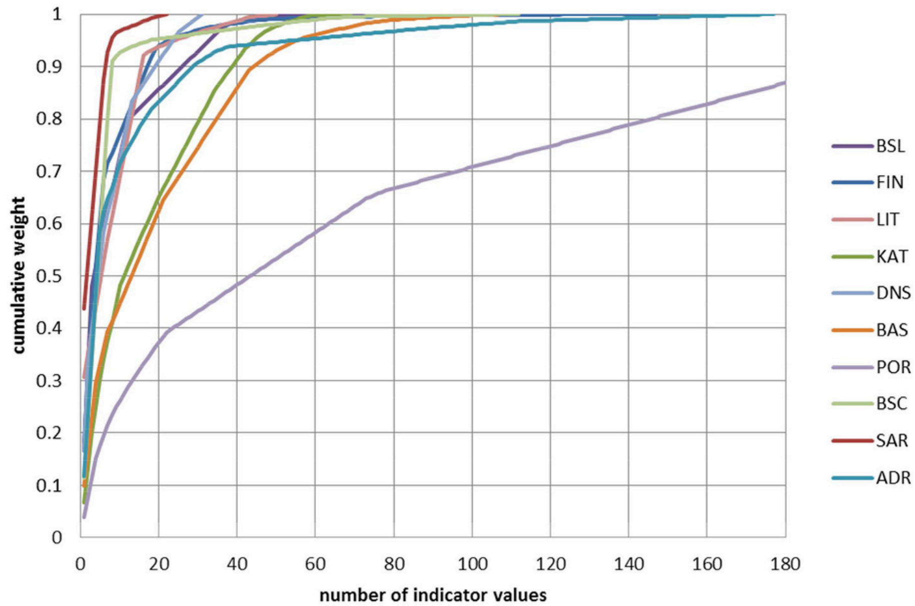

FIGURE 5 | Cumulative contribution to the final assessment (cumulative weight) in relation to the number of indicator values. For each case study, values have been ranked from the most important indicator value (highest weight) to the least important value (lowest weight). The X-axis has been cut; Portuguese case study has a total of 466 indicator values. For case study codes, see **Table 1**.

incorporating the spatial scales issue and the confidence level into the assessment. This could be the reason for which some ecosystem components (e.g., seabirds, mammals, and fishes) have more weight in the final assessment, since they are normally assessed at large scale spatial areas, which have more weight when aggregating (e.g., Saronikos gulf). However, NEAT also includes the possibility to weight indicators differently.

Implications of the Assessment Design

Most of the case study areas lacked indicators regarding one or several biodiversity components and habitats (**Table 1**, **Figure 4**), even those that were deemed important in the area. The lack of indicators stemmed either from lack of monitoring data regarding the area or biological diversity component (e.g., birds, reptiles, pelagic fauna), or from obstacles in the indicator development, including the lack of expert time to develop indicators, or insufficient knowledge about the target levels due to lack of long-term or reference condition data (Hummel et al., 2015). In some cases, more basic ecological research is needed in order to understand the ecological processes well enough to develop indicators. In fact, most of the assessments undertaken until now by member states is more qualitative than quantitative (Hummel et al., 2015), representing a challenge for the assessment.

The habitats and biodiversity components for which no indicators are available potentially affect the final assessment result. It is entirely possible that adding even one indicator that would represent a poorly-represented, large area or habitat, would change the overall assessment for better or for worse. Therefore, in order to make a reliable assessment of the status of the biological diversity, the critical gaps in each assessment case need to be evaluated for their potential to affect the overall result. If such high-leverage gaps exist, the assessment result must be taken with caution.

Different indicator values and spatial assessment units had varying weights in the final assessment result in all of the cases (**Table 3**, **Figure 2**). The differences in the indicator value weights stem from the fact that the default NEAT assessment first assesses the result for each SAU, giving equal weight to each HBT with similar hierarchy, and combines these SAUs hierarchically so that each SAU is given weight according to its area. Therefore, if a SAU has a large surface area and only a small number of indicators per one or several of its habitat types, these indicator values end up contributing strongly to the final assessment.

This emphasizes the importance of the balanced nature of the indicator set, and particularly the reliable assessment of indicators that are used to assess the status of large areas, and particularly their habitats with only few indicators (Feary et al., 2014). Therefore, particular attention should be paid to both the observed value, the boundary values between the classes, and the uncertainty estimation of these most influential indicator values.

The fact that the SAUs are weighted according to their surface area in the default mode of NEAT also emphasizes the need for careful consideration of the definition of the SAUs. Ideally, the SAUs should be defined in the manner that an indicator value defined for a SAU can be expected to reasonably represent all of the SAU. On the other hand, if the assessment area is split into several sub-SAUs and only a fraction of them actually has indicator data, their value will be generalized to represent the whole super-area in the hierarchical assessment anyway.

TABLE 3 | List of the top-five indicator values contributing the most to the overall assessment for each case study.

Spatial Assessment Unit (SAU)	Habitat	Ecosystem component	Indicator name	% contribution to assessment
BARENTS SEA LOFOTEN			***Cumulative contrib. of top-five indicators***	**56**
Norwegian BARENTS SEA–LOFOTEN	sea-ice	Mammals	Harp seal, *Pagophilus groenlandicus* stock size	16.6
Ice-free zone	pelagic	Birds	Kittiwake*, Rissa tridactyla* breeding success over last 5 year	16.4
Seasonal-ice zone	sea-ice	Mammals	Proportion of non-threatened pagophiletic mammals	15
Offshore (ice-free zone)	shelf (muddy sediments)	Benthic Fauna	4 indicators related to macrobenthic fauna: ES100, abundance level, evenness and AMBI	3.7 (each)
GULF OF FINLAND			***Cumulative contrib. of top-five indicators***	**60**
Open sea	Benthic	Benthic Fauna	Average regional diversity	18.4
Gulf of Finland	Pelagic	Fish	Abundance of salmon spawners and smolt	12.5
Gulf of Finland	Pelagic	Fish	Herring, Spawning stock biomass	12.5
Gulf of Finland	Marine (general)	Mammals	3 indicators: Gray seal population growth rate, Gray seal pregnancy rate, Ringed seal population growth rate	8.3 (each)
LITHUANIAN COAST			***Cumulative contrib. of top-five indicators***	**47**
Territorial sea	Benthic	Benthic habitat	Extent of the seabed significantly affected by human activities	31
Territorial sea	Marine (general)	Others	Biopollution level index (invasive species)	4
Territorial sea	Marine (general)	Birds	Abundance of wintering populations of seabirds: (1) Red-throated Diver + Black-throated Loon, (2) Great Crested Grebe (*Podiceps cristatus*), (3) Common Merganser (*Megus merganser*), (4) Velvet Scotter (*Melanitta fusca*), (5) Long-tailed Duck (*Clangula hyemalis*), and (6) Common Goldeneye (*Bucephala clangula*)	4 (each)
KATTEGAT DK			***Cumulative contrib. of top-five indicators***	**30**
KATTEGAT, central parts	Marine (general)	Birds	Fulmar winter abundance (encounter rate)	6.6
KATTEGAT, central parts	Marine (general)	Birds	Kittiwake winter abundance (encounter rate)	6.6
KATTEGAT, central parts	Marine (general)	Birds	Guillemot winter abundance (encounter rate)	6.6
KATTEGAT, central parts	Benthic	Benthic Fauna	BQI	5
kATTEGAT, central parts	Benthic	Benthic Fauna	DKI	5
DUTCH NORTH SEA			***Cumulative contrib. of top-five indicators***	**21**
Dutch EEZ	Benthic	Benthic Fauna	Benthic invertebrates total number of species	16.7
Dutch EEZ	Muddy deep bottom	Benthic Fauna	3 indicators related to typological group sensitive to seafloor physical impact, based on (1) density, (2) biomass, (3) number of species	1.1 (each)
Dutch EEZ	Muddy deep bottom	Benthic Fauna	4 indicators related to typological group highly sensitive to seafloor physical impact, based on (1) density, (2) biomass, (3) number of species	1.1 (each)
BASQUE EEZ			***Cumulative contrib. of top-five indicators***	**33**
Offshore waters (>200 m depth)	Benthic	Benthic habitat	Seabed affected by human activities	9.9
Offshore waters (>200 m depth)	Pelagic	Birds	Biological value Seabirds	6.6
Offshore waters (>200 m depth)	Pelagic	Mammals	Biological value Mammals	6.6
Offshore waters (>200 m depth)	Pelagic	Phytoplankton	Eutrophication indicator: Chlorophyll a, 90th percentile	6.6
Offshore waters (>200 m depth)	Sedimentary	Benthic Fauna	2 indicators: (1) M-AMBI, (2) AMBI	3.3 (each)
Offshore waters (>200 m depth)	Sedimentary	Fish	Biological value Demersal Fish	3.3

(Continued)

TABLE 3 | Continued

Spatial Assessment Unit (SAU)	Habitat	Ecosystem component	Indicator name	% contribution to assessment
PORTUGUESE CONTINENTAL SUBDIVISION			***Cumulative contrib. of top-five indicators***	**17**
Continental_A2_600	Marine (general)	Birds	Biological Value Marine Birds	3.9
Continental_A2_600	Pelagic	Zooplankton	Biological Value Zooplankton	3.9
Continental_B4_600	Sedimentary	Benthic Fauna	Biological Value Benthic communities	3.7
Continental_B4_600	Marine (general)	Birds	Biological Value Marine Birds	3.7
Continental_B5_600	Marine (general)	Birds	Biological Value Marine Birds	2.2
COASTAL BLACK SEA			***Cumulative contrib. of top-five indicators***	**57**
Black Sea coastal	Pelagic	Fish	4 indicators: (1) Mean length of *Sprattus sprattus*, (2) Catch/biomass ratio of *S. sprattus*, Biomass of *S. sprattus*, (4) Sexually mature specimen of *S. sprattus*	11.4 (each)
Black Sea coastal	Benthic	Fish	4 indicators: (1) Mean length of *Scophtalmus maximus*, (2) Catch/biomass ratio of *S. maximus*, (3) Biomass of *S. maximus*, (4) Sexually mature specimen of *S. maximus*	11.4 (each)
SARONIKOS GULF			***Cumulative contrib. of top-five indicators***	**62**
Saronikos Gulf	Marine (general)	All Taxa	CIMPAL index (alien species)	44
Saronikos Gulf	Pelagic	Reptiles	% loss of spawning areas of sea turtle *Caretta caretta*	4.4
Saronikos Gulf	Pelagic	Fish	4 indicators: Fisging mortality for (1) *Engraulis encrasicolus*, (2) *Sardina pilchardus*, (3) *Merluccius merluccius*, (4) *Mullus barbatus*	4.4 (each)
Saronikos Gulf	Pelagic	Mammals	% Threatened mammals	4.4
Saronikos Gulf	Pelagic	Fish	% Threatened sharks	4.4
Saronikos Gulf	Pelagic	Fish	% of stocks that meet GES based on fishing mortality	4.4
Saronikos Gulf	Pelagic	Fish	% of stocks that meet GES based on reproductive capacity	4.4
Saronikos Gulf	Pelagic	Fish	% of stocks that meet GES based on reproductive capacity and biomass indices	4.4
ADRIATIC SEA			***Cumulative contrib. of top-five indicators***	**58**
Adriatic sea	Pelagic	Mammals	*Tursiops truncatus*, distributional range	11.7
Adriatic sea	Pelagic	Mammals	*Stenella coeruleoalba*, distributional range	11.7
Adriatic sea	Pelagic	Mammals	*Grampus griseus*, distributional range	11.7
Adriatic sea	Pelagic	Mammals	*Ziphius cavirostris*, distributional range	11.7
Adriatic sea	Pelagic	Mammals	*Tursiops truncatus*, abundance	11.7

In case of equal contribution of several indicator values, all the indicator values are given. The contribution to the overall assessment (in %) of each indicator value is given. Numerical values are rounded. ES100, expected number of species in 100 individuals; AMBI, AZTI's Marine Biotic Index; BQI, Benthic Quality Index; DKI, Danish Index; CIMPAL, Cumulative IMPacts of invasive ALien species; M-AMBI, multivariate AMBI; GES, good environmental status.

In NEAT, it is possible to weight the SAUs according to their perceived ecological relevance instead of their surface area; for example, biodiversity hotspots, important reproduction areas, marine protected areas, etc., could be given a higher weight than their area alone would imply. In this study, this option was not used in any of the case studies.

Uncertainty of the results is assessed based on Monte Carlo simulations, using the observed value as mean and the standard error value as the standard deviations, assuming a Gaussian distribution (Carstensen and Lindegarth, 2016). Based on these simulations, NEAT determines how often the sampled value falls into each of the five classes, and this distribution is reported. Therefore, the standard error values assigned to the indicators play a major role in the uncertainty associated with the final assessment result. This emphasizes the importance of careful evaluation of the standard deviation, particularly with indicators that have a high weight in the assessment.

Evaluation of the Assessment Results

There are other tools to assess the status of marine systems, e.g., the Ocean Health Index (OHI; Halpern et al., 2012). This index has different concept and a much broader spatial scale, and a comparison between NEAT and OHI results (BD values presented in Table S6 in Selig et al., 2013) shows that the results are quite different (**Table 5**).

TABLE 4 | Number of indicator values per ecosystem component, and the relative weight of ecosystem components in each case study.

	Number of indicator values												% of total weight													
	Phytoplankton	Zooplankton	Benthic Veg	Benthic Fauna	Fish	Birds	Mammals	Reptiles	Pelagic Fauna	All Taxa	Water column habitat	Benthic habitat	Phytoplankton (%)	Zooplankton (%)	Benthic Veg (%)	Benthic Fauna (%)	Fish (%)	Birds (%)	Mammals (%)	Reptiles (%)	Pelagic Fauna (%)	All Taxa (%)	Water column habitat (%)	Benthic habitat (%)
Norwegian Barents Sea–Lofoten	1	4	3	37	9	14	6	0	0	0	0	0	0	3	0	20	14	28	34					
Gulf of Finland	53	2	34	46	4	2	3	0	0	0	3	0	10	4	2	20	30	3	25				6	
Lithuanian marine waters	3	6	1	1	6	19	0	0	0	3	8	3	4	9	0	0	1	29				5	20	32
Kattegat	5	1	25	9	11	3	1	0	0	0	14	0	2	0	26	14	16	20	1				21	
Dutch North Sea	2	2		19	6	1	1	0	0	0	0	0	7	7		33	45	4	4					
Basque Coast	8	5	5	19	42	6	6	0	0	0	12	6	8	1	0	10	54	8	8				1	11
Portuguese continental subdivision	44	42		27	108	44	0	0	177	24	0	0	8	9		8	29	21			25	1		
Black Sea coast (Large Varna Bay)	36	24	4	12	12	0	0	0	0	0	20	4	2	1	2	2	92						1	0
Saronikos Gulf	0	0	2	12	8	0	1	1	0	1	4	0			3	3	26		9	9		44	6	
Adriatic Sea	3	0	0	100	0	0	30	0	0	0	37	7	0			8			89				3	0

The OHI tends to give a more reduced range of status values (74–97) than those provided by NEAT (0.37–0.69) for these areas. The OHI does not provide a GES/non-GES status, but in general provides higher values than those by NEAT. The OHI study (Selig et al., 2013) has been applied globally, and includes a large variety of worldwide cases with great differences in setting and problems. In that context, e.g., the Mediterranean and the Baltic Sea seem to be in a (seemingly more homogenous) better state than e.g., waters around Africa or Indonesia and Philippines.

An interesting observation is that there is a negative rather than a positive correlation between these results, and those areas ranked low in NEAT (such as the Gulf of Finland and Kattegat) get high scores in OHI, while the best-scoring area in NEAT (Basque EEZ) gets lowest score in OHI (**Table 5**). This discrepancy is partly due to the fact that the OHI scores are given by country, thus covering larger areas than the case studies assessed here with NEAT. Therefore, the local status of a case study area may be masked by the results from the rest of the country in OHI. The NEAT results are reported here for the entirety of each of the case study areas, but where the case study area includes smaller SAUs, the results can be viewed for each of them separately as well, yielding even a more detailed geographical resolution.

Another factor possibly contributing to this discrepancy is the use of different indicators; the OHI assessment used publically available data with little local/regional detail, which can vary the final assessment when applying to regional scales (Halpern et al., 2014), while the current NEAT assessment used indicators specifically designed for marine status assessment. The species scores of OHI focused on the extinction risk of marine species (Selig et al., 2013), while the indicators in the NEAT assessments included a wider spectrum of indicators of species status. The OHI habitat scores were based on condition estimates of mangroves, coral reefs, seagrass beds, salt marshes, sea ice, and subtidal soft-bottom (Selig et al., 2013) while the NEAT assessments were tailored for each area.

The NEAT assessment results were in most cases in line with previous regional/local assessments, understanding, or known pressure gradients (**Table 1**, **Figure 4**). For example, The Baltic Sea biodiversity has been assessed by HELCOM (2009a, 2010) to be in poor to bad status in all of the three Baltic case study areas included in this analysis (Gulf of Finland, Lithuanian marine waters, Kattegat), being similar to the NEAT results but not to the OHI assessments. The difference between the NEAT and OHI results in these cases is probably largely due to eutrophication, which is documented to be major pressure threatening the ecosystem functioning of the Baltic Sea (HELCOM, 2009b, 2010). While it is reflected in the status of phytoplankton and water column habitats, and also affects the higher trophic levels of the food web (Österblom et al., 2007) and the seafloor (Karlson et al., 2002), it is not likely to be strongly reflected in the extinction threat of marine species (used in OHI), although it does affect the habitat scores, particularly seagrasses (Table S1 in Selig et al., 2013).Another factor affecting the discrepancy in the case of Finland is that the Gulf of Finland area has poorer biodiversity status than the Finnish marine waters on average (HELCOM, 2010).

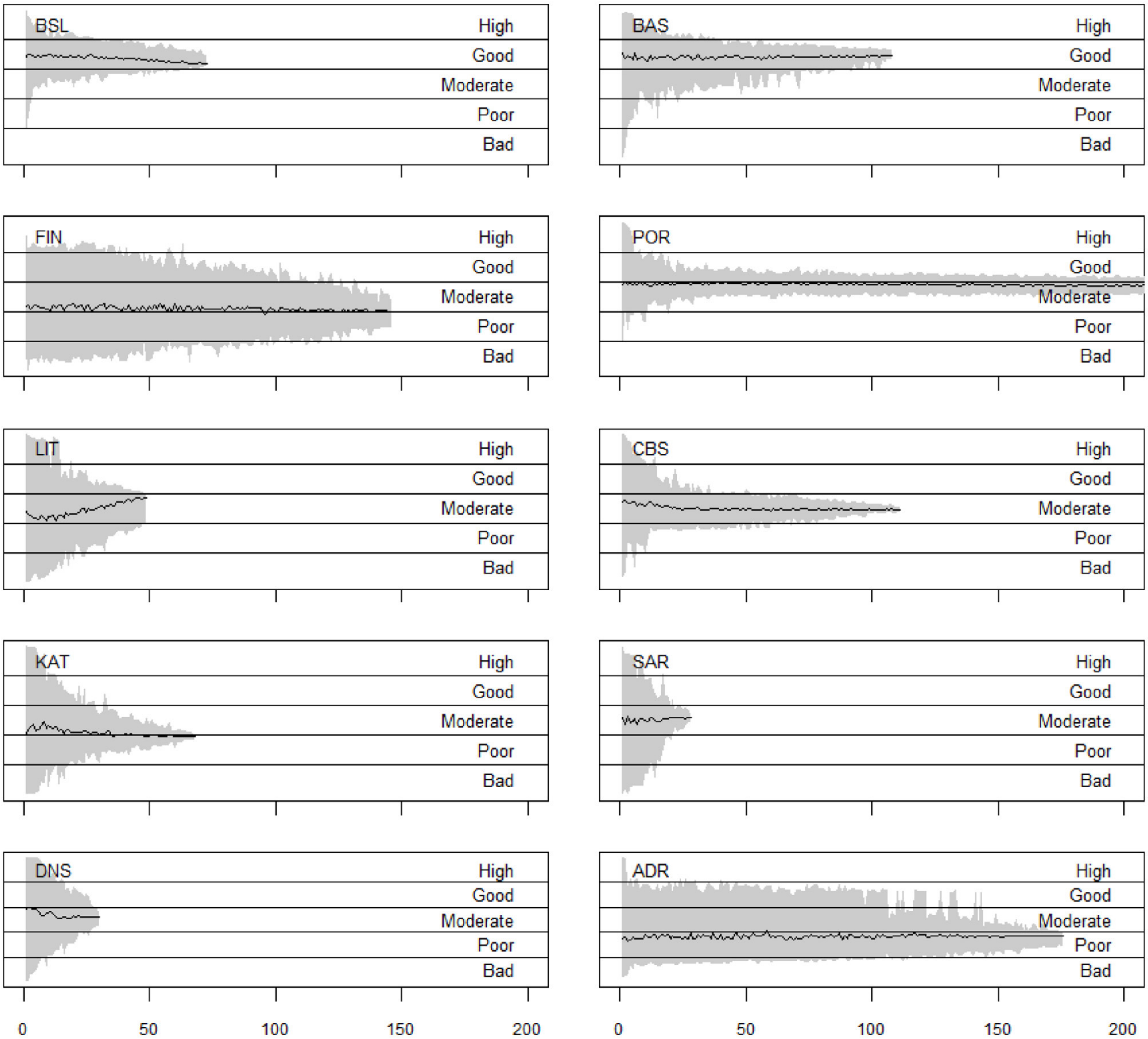

FIGURE 6 | Variation of the overall assessment results in the case studies, if only a subset of the indicator values is taken into account. The x axis indicates how many indicators are included into the assessment, and the gray area spans the assessment result values that emerged. The black line shows the mean assessment result across the 100 runs conducted for each number of indicator values. Note that the x axis goes only up to 200 indicator values; the Portuguese continental subdivision study included 466 indicator values in total.

In the North Sea, fishing is considered the main pressure, and the results show fish to be the ecosystem component in poorest status; the other assessed ecosystem components (birds, mammals, benthic fauna, and phytoplankton) were assessed to be in GES, with the exception of zooplankton that showed sub-GES (moderate) status (**Figure 4**). The Black Sea Coast case results obtained in this study also corresponded very well to known pressure gradients, such as nutrient enrichment affecting the status of the plankton community (**Figure 4**). Phytoplankton and benthic vegetation assessments correspond to category "poor" in the Varna Bay itself (Dencheva and Doncheva, 2014; Moncheva et al., 2015) as the most affected by anthropogenic pressure among the BSC sub-SAUs (Shtereva et al., 2012). The lowest benthic fauna score is also found there, which is fully in compliance with recently published results (National Report on the State and Protection of the Environment in Bulgaria, 2014). Similarly, the Basque area, which was previously assessed as being in good status, using a different methodology (Borja et al., 2011) also results in good status after applying NEAT; only mammals were assessed to be in sub-GES status (**Figure 4**).

In Saronikos Gulf the assessment results correspond to the ecological status categorization according to the WFD which is

TABLE 5 | Comparison of the biodiversity assessments obtained using the Ocean Health Index (OHI; data from Selig et al., 2013) and the Nested Environmental status Assessment Tool (NEAT) (this study) in the countries for which NEAT has case studies.

Country (Case study)	Status (NEAT)	Biodiversity score (OHI)
Norway (Barents Sea)	0.646	90
Finland (Finland)	0.401	97
Lithuania (Lithuania)	0.583	89
Denmark (Kattegat)	0.394	93
Netherlands (North Sea)	0.523	85
Spain (Basque Country)	0.689	74
Portugal	0.566	83
Bulgaria (Bulgaria)	0.495	87
Greece (Saronikos Gulf)	0.520	91
Italy/Croatia (Adriatic)	0.370	86/89

poor in the sewage outfall area and moderate in the inner central gulf (Simboura et al., 2014, 2015, 2016). Aliens, fish including threatened sharks, and mammals contributed to the moderate status seen for the outer Saronikos and overall Saronikos. In general, the respective assessment results, although not definitive, are in line with pertinent studies (Frantzis, 2009; Katsanevakis et al., 2013; Papaconstantinou, 2014; Vasilakopoulos et al., 2015; Zenetoσ et al., 2015; Simboura et al., 2016) regarding the Greek marine waters. The Saronikos Gulf result obtained in this analysis was lower than the OHI assessment of the Greek waters, which was to be expected, as the Gulf is intensely exploited.

Results from the Norwegian part of the Barents sea indicated a general good status, which is in accordance with indicators of fish status on exploited large marine ecosystems (Kleisner et al., 2014; Coll et al., 2015), the report on the Barents Sea management plan (Sunnana et al., 2010) and the work from Certain et al. (2011). Nevertheless, several indicators indicated potentially degraded situations both in the coastal area and in the area of seasonal ice presence: (1) Along northern Norway coast, the current extent of kelp forest, an important component of fjords ecosystem and coastal landscape, cannot be considered as good in northern Norway. Kelp forests along the Norwegian and Russian coast were indeed dramatically grazed during the early 1970s and replaced by barren grounds dominated by sea urchins (Norderhaug and Christie, 2009). Though a progressive northward recovery of kelp forests extent is observed, its recovery status is still partial in northern Norway (Sivertsen, 2006; Rinde et al., 2014). (2) In northernmost part of the Barents sea, sea-ice extent is undergoing a particularly dramatic decrease (Parkinson et al., 1999) with a significant decrease rate of −3.5% per decade of winter ice extent (Sorteberg and Kvingedal, 2006) as a response to climate warming (Boitsov et al., 2014). This dramatic loss of habitat has consequences on the associated communities (Kovacs et al., 2011) as well as in the functioning of the Barents sea ecosystem as a whole (Wassmann et al., 2006). The growing evidence of impacts of climate change on this area rises the issue of exogenic unmanaged pressures on this system and the issue of shifting baselines for the definition of target values. In addition, there are still no indicators of the impact of trawling activities included in this assessment (see however Jørgensen et al., 2016).

For the Portuguese coast, the initial assessment officially provided in the scope of the MSFD (MAMAOT, 2012), presented a general environmental quality status higher than the NEAT results calculated in this study. This may be partly due to the fact that the present assessment did not include some special areas with a higher degree of protection (such as Berlengas' Marine Reserve and Professor Luiz Saldanha's Marine Park or Goringe Seafloor). These areas, which have restricted access by the public, are important for marine high trophic level species (e.g., marine birds, mammals), some of which were not included in the present assessment. Due to inconsistencies in the data (now being improved by projects such as MARPRO—Conservation of Marine Protected Species in mainland Portugal, http://marprolife.org), marine mammals, reptiles and benthic vegetation were not included in the current NEAT assessment, which may also contribute to the lower environmental quality results achieved by NEAT. The higher result reported by the OHI may be related to the methodology used for the scores' calculation, and may reflect more specifically the trend than the present environmental status.

An exception to the good correspondence between the current and previous assessments is the Adriatic Sea, where the assessment provided by NEAT appears too low considering the current trends, also reported in the scientific literature, and available information from expert opinions (Coll et al., 2010; Bastari et al., 2016). Despite the historical impacts on this shallow water basin, the Adriatic Sea is still characterized by a wide diversity of habitats, including rocky and soft bottoms, large estuaries and lagoons, seagrass meadows and in, its southern part, also deep-water environments. The habitat richness is reflected by a high biodiversity (Coll et al., 2012; Micheli et al., 2013b), with approximately 49% of the species described for the Mediterranean Sea (Boudouresque et al., 2009; UNEP, 2015) and a variety of endemic species (e.g., 18% of the endemic fish species of the Mediterranean; UNEP/MAP-RAC/SPA, 2015). Human activities and multiple stressors, and in particular bottom trawling, hydraulic dredging and habitat loss, are certainly still impacting the Adriatic Sea (Micheli et al., 2013a; Pusceddu et al., 2014). However, the overall environmental condition is not worsening with respect to the past decade. Eutrophication and dystrophic crises, related to the high nutrient discharge from the Po River combined with an alteration in water circulation, have caused hypoxia, anoxia and massive mucilage events, with consequent mortality of the benthic organisms, but the frequency of these events decreased significantly (or even disappeared) in the last decade (Degobbis et al., 2000; Danovaro et al., 2009). Thus, we hypothesize that the assessment of the environmental status obtained by using NEAT can be affected by the number and typology of data included in the specific exercise. An improvement of the number and type of the biological indicators (e.g., species or ecosystem functioning) could be crucial to obtain a more realistic classification of the marine environmental health of the Adriatic Sea.

Birds and mammals were found to be in poor status in many of the case study areas. This reflects the fact that seabirds are indeed considered as more threatened than any other comparable groups of bird species in general and display a faster trend of decline than other bird species during the last decades (Croxall et al., 2012). In addition, using IUCN Red list categories, it has been evidenced that, among seabirds, pelagic species of seabirds are disproportionately more threatened than coastal resident or coastal non-breeding visitor species (Croxall et al., 2012). Pelagic seabirds are particularly sensitive to disturbance as most species lay only a single egg, adults do not reproduce every year and usually reproduce several years after reaching sexual maturity (Furness and Camphuysen, 1997). Most seabird species display very large home range and thus integrate the state of the environment and impacts of pressures over larger scale.

The conservation status of marine mammals is of particular concern with an estimated proportion of threatened species ranging worldwide between 23 and 61% of species (Schipper et al., 2008). The North Atlantic region, which includes several of the cases studied here, is one of the areas where the proportion of threatened marine mammals is the highest, as shown by the low quality values in Barents Sea, Kattegat, and Basque case studies (**Figure 4**). The main reported threats explaining the bad status of marine mammals are a long history of harvesting, accidental mortalities through bycatch and collisions with vessel as well as a very large panel of pollutions (from sound pollution to contaminants and marine debris) and climate change (Schipper et al., 2008). The sensitivity of these species to changes in their environment might be related to their very slow population dynamics, low densities in correlation with their large body-size (Cardillo et al., 2008). Those life traits are also related with relatively large home range. As a consequence, indicators of marine mammals are usually measured over large scale, and they are difficult to monitor with precision, leading to higher uncertainty on many indicators (Taylor et al., 2007).

In two of the areas (Lithuania and Basque coasts), the indicator contributing the most to the final assessment was "the extent of the seabed significantly affected by human activities," which is a direct indicator of pressure. This is interesting since some authors (e.g., Borja et al., 2013) have supported the use of pressures instead of assessing the environmental status, if there are not enough indicators. This should be done under the premise that if an area has no obvious pressures then any changes in the area must be due to natural changes which are outside the control of management and vice versa.

Sensitivity Analysis

The sensitivity analysis results show differences among the case studies in terms of how many indicator values are needed before the assessment results will show approximately the same results regardless of which indicator values are selected into the assessment (**Figure 6**). This implies that there is no universally sufficient number of indicator values needed to make a reliable assessment, but that the number varies among case studies. No clear patterns could be found among the 10 cases evaluated in this study that would indicate a number of indicator values of biodiversity components that can be considered sufficient regardless of the case study and its structure.

The variation in the assessment result depends on the set of indicator values that is available for the assessment. If the indicator values are close to each other, i.e., all indicating similar status, the variation in the results is naturally smaller. In contrast, if the different indicator values indicate very different status, e.g., some areas or biodiversity components are in good status while others are in bad, this naturally incurs a larger variation when a subset of these variables are selected, as e.g., in the Gulf of Finland.

These observations lead to the conclusions that if there is variation among the status of the geographical or biodiversity components in the study area, all of them should be covered by indicators if possible. Particularly the inclusion of high-leverage indicator values, i.e., those that have high weight and whose value differs from the overall mean, can change the assessment result. Therefore, the careful evaluation of the value and class limits of these indicators should be a priority.

CONCLUSIONS

The structured assessment forces us to critically evaluate the available indicator set in terms of ecological and spatial representativeness of each indicator. This framework highlights the gaps in the assessment as well as those parts that are well-represented by current monitoring and available indicators. This, in turn, helps in determining the best way to improve the quality of the assessment: (i) via developing additional indicators to fill in the gaps within the ecosystem approach (i.e., if not all the important trophic levels of key species/ groups are covered in the existing indicator set), (ii) working to determine the optimal SAU for different categories of indicators that are targeted to assess various trophic levels and functions in the food web, as well as the HBT classification for each area, and (iii) working toward improving specificity, robustness, and pressure relevance of the indicators and enabling estimation of their standard errors.

The development of NEAT and this extensive testing with 10 case studies in very different European marine areas offers insight both to the status of the marine waters and to the state-of-the art of the available indicator assemblages as well as the development needs of the marine biological diversity assessment. The application of the tool will make the improvement and harmonization needs of the assessments visible and pave the way toward a harmonized assessment across large geographical scales.

In conclusion, we propose the following recommendations for the best practice in performing the environmental status assessment using NEAT:

- Careful attention needs to be paid particularly to the current status and class boundaries of the indicators that cover large geographical areas (such as mobile birds and mammals), as they tend to carry a lot of weight in the final assessment.
- In order to make the assessment comparable between the different sub-regions and areas in the regional seas and provide a harmonized assessment among the regional seas, the design of the assessment needs to be harmonized. Attention must be paid to the selection of ecosystem components, and definition

of size and hierarchy of the spatial assessment units as well as the definition of habitats.
- Consider the possibility of using different weighting for the individual indicator values, if that is ecologically more justified than using the weight based on the spatial area and habitat weighting.
- Contextualize the outputs on the basis of existing data. Different ecosystem components may present quite different data coverage, frequency, and data quality for the evaluation, and that may be reflected in the results. Consider carefully the standard deviation assigned to the indicators, but also consider how well the available indicators represent the ecosystem component and/or area as a whole.
- Consider not only the overall assessment, but the partial assessments (e.g., biological components or MSFD descriptors), as partial assessments can contribute to increased understanding of results and defining management measures for specific issues or areas.

AUTHOR CONTRIBUTIONS

LU, HB, and AB conceived the paper. The following partners provided the case studies: HB, SC (Barents Sea–Lofoten), LU, VK, HN (Gulf on Finland), GS (Lithuanian marine waters), CM, JA, JC (Kattegat), OB (Dutch North Sea), AB, MU (Basque Coast), JN (Portuguese continental subdivision), SM (Black Sea Coast), NP, MP, NS (Saronikos Gulf), RD, LC, AC, SB (Adriatic Sea). LU wrote the first draft. TB and CM contributed to the calculations and sensitivity analyses. LU, HB, AB, SC, AH, and MU contributed largely to the introduction and discussion. All authors contributed to the last draft and to the discussions.

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SUPPLEMENTARY MATERIAL

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Mapping ecosystem services provided by benthic habitats in the European North Atlantic Ocean

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The mapping and assessment of the ecosystem services provided by benthic habitats is a highly valuable source of information for understanding their current and potential benefits to society. The main objective of this research is to assess and map the ecosystem services provided by benthic habitats in the European North Atlantic Ocean, in the context of the "Mapping and Assessment of Ecosystems and their Services" (MAES) programme, the European Biodiversity Strategy and the implementation of the Marine Strategy Framework Directive (MSFD). In total, 62 habitats have been analyzed in relation to 12 ecosystem services over 1.7 million km^2. Results indicated that more than 90% of the mapped area provides biodiversity maintenance and food provision services; meanwhile, grounds providing reproduction and nursery services are limited to half of the mapped area. Benthic habitats generally provide more services closer to shore—rather than offshore—and in shallower waters. This gradient is likely to be explained by difficult access (i.e., distance and depth) and lack of scientific knowledge for most of the services provided by distant benthic habitats. This research has provided a first assessment of the benthic ecosystem services on the Atlantic-European scale, with the provision of ecosystem services maps and their general spatial distribution patterns. Regarding the objectives of this research, conclusions are: (i) benthic habitats provide a diverse set of ecosystem services, being the food provision, with biodiversity maintenance services more extensively represented. In addition, other regulating and cultural services are provided in a more limited area; and (ii) the ecosystem services assessment categories are significantly related to the distance to the coast and to depth (higher near the coast and in shallow waters).

Keywords: ecosystem service, benthic habitat, Regional Seas, Marine Strategy Framework Directive, habitat classification

INTRODUCTION

Functioning ecosystems are essential for maintaining the oceans in a healthy state (Tett et al., 2013). While being healthy, they provide numerous and diverse goods and services that contribute "for free" to the general well-being and health of humans (Van Den Belt and Costanza, 2012). The "ecosystem goods and services" term integrates two concepts: (i) the ecosystem goods, which represent marketable material products that are obtained from natural systems for human use, such as food and raw materials (De Groot et al., 2002); and (ii) ecosystem services, which refers to all "the conditions and processes through which natural ecosystems, and the species that make them up, sustain and fulfill human life" (Daily, 1997). The latter are not directly marketable services, and include nutrient recycling, biodiversity maintenance, climate regulation or cultural and esthetic services (Costanza et al., 1997). Ecosystem services occur at multiple spatial scales; from the global, such as climate regulation, primary production, and carbon sequestration, to a more regional or local scale, such as coastal protection and leisure.

Previous studies show that coastal ecosystem services provide an important portion of the total contribution of ecosystem services to human welfare (Pimm, 1997; Pearce, 1998). Costanza et al. (1997) showed that, while the coastal zone only covers 8% of the world's surface, the services that this zone provides are responsible for approximately 43% of the estimated total value of global ecosystem services. Despite our dependence on biodiversity and ecosystem services, population expansion and economic growth are leading to increasing anthropogenic pressures on coastal areas (Wilson et al., 2013) and consequently, to a decreasing supply of ecosystem services worldwide (Costanza et al., 2014). Recognizing that human pressures directly impact on ecosystem services and that in turn, ecosystem services directly benefit human well-being, they have sparked interest amongst coastal planners and have led to the integration of ecosystem services in conservation management measures (Cimon-Morin et al., 2013).

Due to the above-mentioned reasons, ecologists, social scientists, economists and environmental managers are increasingly interested in assessing the economic values associated with the ecosystem services of coastal and marine ecosystems (Bingham et al., 1995; Costanza et al., 1997; Daily, 1997; Farber et al., 2002; Liquete et al., 2013a). Different approaches and frameworks have been proposed to identify, define, classify and quantify services provided by marine biodiversity (MEA, 2003; Ten Brink et al., 2009; Cices, 2013; Liquete et al., 2013a). Neither of these approaches being a straight forward one; the accurate estimation of the values of services, and in particular their temporal and spatial variation, is relatively new and has not been extensively researched (Schägner et al., 2013).

Indeed, the complexity of the processes and functioning of marine ecosystems, and their highly dynamic nature, translates into the absence or low resolution of spatially explicit information. Furthermore, the deep sea, and in particular benthic habitats, is mostly lacking in ecosystem services assessments (Armstrong et al., 2012; Thurber et al., 2013). Due to these limiting factors, there are few published studies, and they mainly focus on food production, such as fisheries, with other services receiving minor attention (Murillas-Maza et al., 2011; Liquete et al., 2013a; Seitz et al., 2014). Mapping and assessing ecosystem services may help to overcome such hindrances. Maps not only enable the characterization of current benefits that services provide to society, but also the adoption management measures that guarantee their future provision and contribution to human welfare (Egoh et al., 2012).

To date, several habitat mapping efforts have been carried out at different spatial and temporal resolutions (Liquete et al., 2013a). Within Europe, Mapping and Assessment of Ecosystems and their Services (MAES) is one of the keystones of the EU Biodiversity Strategy to 2020 (Maes et al., 2013). This strategy demands Member States to map and assess the state of ecosystems and their services in their national territory (including their marine waters) with the assistance of the European Commission. The results of this mapping and assessment should support the maintenance and restoration of ecosystems and the services they provide (Maes et al., 2013). It will also contribute to the assessment of the economic value of ecosystem services, and promote the integration of these values into accounting and reporting systems at EU and national level by 2020. The results are expected to be used to inform policy decision makers and policy implementation in many fields, such as nature and biodiversity, territorial cohesion, agriculture, forestry, and fisheries. Outputs can also inform policy development and implementation in other domains, such as transport and energy (Maes et al., 2013). For example, the Marine Strategy Framework Directive (MSFD, 2008/56/EC) requires the availability of ecosystem services valuation for the assessment of the environmental status and to define the measures that make sustainable human activities at sea (Cardoso et al., 2010). Hence, according to the MSFD, the assessment of the environmental status should be undertaken for the Exclusive Economic Zone (EEZ) of the Member States within the four European Regional Seas: North Eastern Atlantic, Baltic, Mediterranean, and Black Seas.

In this context, the objectives of this research were: (i) the qualitative assessment and mapping of the ecosystem services provided by benthic habitats within the European North Atlantic Ocean; and (ii) to determine if ecosystem services assessment categories are related to the habitat distance to the coast and depth. The analysis was based on available cartographic information and ecosystem services assessment, focusing on the benefits that they provide in the Regional Seas and sub-regions defined by the MSFD.

MATERIALS AND METHODS

The implementation of ecosystem services valuation involves two dimensions: (i) a biophysical assessment of services supply; and (ii) a socio-economic assessment of the value per unit of services (Schägner et al., 2013). Within this investigation, we focused only on the first approach of trying to map and assess the ecosystem services provided by benthic habitats at the European North Atlantic Ocean scale. This is because the economic value of the services is still poorly known, needing comprehensive data supply, which the results from this investigation can provide.

GEOGRAPHIC AREA

For this investigation, the North Eastern Atlantic was selected. According to MSFD, the North Eastern Atlantic Ocean is divided into four sub-regions: Greater North Sea, Celtic Seas, Bay of Biscay and Iberian coasts, and Macaronesia (**Figure 1**). It should be noted that at the time of this investigation, no official geographical delimitations of the sub-regions were adopted, and therefore, they were defined according to the EEZs. The total area of the European North Atlantic Ocean covered by the MSFD is 4,540,025 km^2, which corresponds to the EEZ of 10 European Member States and part of Norway (**Figure 1**).

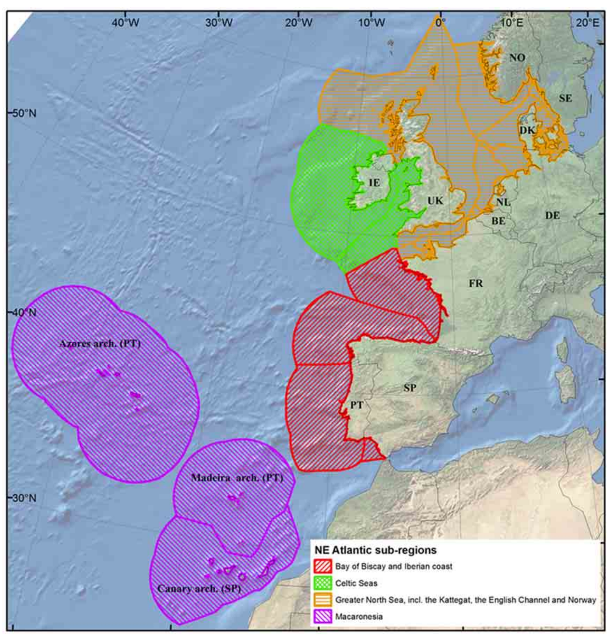

FIGURE 1 | European North Atlantic Ocean sub-regions. Spatial limits are based on the Marine Strategy Framework Directive and Exclusive Economic Zone of the countries located in each sub-region. BE, Belgium; DK, Denmark; FR, France; DE, Germany; IE, Ireland; NL, Netherlands; NO, Norway; PT, Portugal (including Azores archipelago and Madeira archipelago); SP, Spain (including Canary archipelago); SE, Sweden; and UK, United Kingdom.

BACKGROUND INFORMATION USED IN THE ANALYSIS

In order to proceed with the mapping of ecosystem services, main bathymetric and habitat data were obtained from the following sources:

- EMODnet—European Marine Observation and Data Network [http://www.emodnet-hydrography.eu/; European Commission; Directorate-General for Maritime Affairs and Fisheries (DG MARE)]. EMODnet-Hydrography portal provides hydrographic data collated for a number of sea regions in Europe. Bathymetric information was available as Digital Elevation Model at 500 m (c.a. 0.0042°) grid resolution.
- EUSeaMap—Mapping European seabed habitats (http://jncc.defra.gov.uk/page-6266). EUSeaMap is a broad-scale modeled habitat map built in the framework of MESH (Mapping European Seabed Habitats) and BALANCE (Baltic Sea Management—Nature Conservation and Sustainable Development of the Ecosystem through Spatial Planning) INTERREG IIIB-funded projects. EUSeaMap covers over 2 million km^2 of European seabed (Cameron and Askew, 2011). This information layer was available in polygon format.
- MeshAtlantic project (www.meshatlantic.eu; Atlantic Area Transnational Cooperation Programme 2007–2013 of the European Regional Development Fund). It covers over 356,000 km^2 of seabed habitats of the European North Atlantic Ocean produced 250 m (c.a. 0.0027°) grid resolution. This information layer was available in polygon format (Vasquez et al., in press).

DIGITAL ELEVATION MODEL

To produce the digital elevation model information layer, bathymetric information from MeshAtlantic and EMODnet was mosaicked. The information on this layer enabled the investigation of the depth distribution of benthic habitats in the sub-regions of the mapped areas.

BENTHIC HABITATS INFORMATION

For practical purposes of mapping and assessment (i.e., data availability) this investigation focused on "benthic habitats," as a means to assess the provision of ecosystem goods and services.

Habitats were classified according to EUNIS (European Union Nature Information System) habitat classes (Davies et al., 2004). The EUNIS habitat classification aims to provide a common European reference set of habitat types to allow the reporting of habitat data in a comparable manner for use in nature conservation (e.g., inventories, monitoring, and assessments) (Davies and Moss, 2002; Davies et al., 2004; Galparsoro et al., 2012). The classification is organized into hierarchical levels (EUNIS habitat type hierarchical view is available at http://eunis.eea.europa.eu/habitats-code-browser.jsp). The present version of the classification starts at level 1, where "Marine habitats" are defined, up to level 6, by using different abiotic and biological criteria at each level of the classification. For seabed habitats for which EUNIS classes were not defined, underwater features defined under EUSeaMap (e.g., infralittoral seabed) were retained.

Habitat maps were transformed into raster format and mosaicked to obtain a total broad-scale habitat map. In overlapping cells, MeshAtlantic habitat classes were kept, according to the criteria that this represents the most recent information. The mapped area outside EEZ of Ireland was excluded from the later analysis, in order to make results comparable among different countries, in which only EEZ areas were included.

Finally, to analyse the spatial distribution of benthic habitats (in terms of their distance to shore) and therefore, that of the ecosystem services that they provide, the distance of each cell, assigned to each habitat type, to the nearest coastline point was estimated using Euclidean distance algorithm, in a Geographic Information System (GIS).

ECOSYSTEM SERVICES ASSESSMENT

In total, twelve ecosystem services were considered in this investigation: (i) Food provision; (ii) Raw materials (biological) (incl. biochemical, medicinal, and ornamental); (iii) Air quality and climate regulation; (iv) Disturbance and natural hazard prevention; (v) Photosynthesis, chemosynthesis, and primary production; (vi) Nutrient cycling; (vii) Reproduction and nursery; (viii) Maintenance of biodiversity; (ix) Water quality regulation and bioremediation of waste; (x) Cognitive value; (xi) Leisure, recreation and cultural inspiration; and (xii) Feel good or warm glow.

Ecosystem services were classified into: (i) Provisioning services (i.e., 1 and 2 from the above list); (ii) Regulating services (i.e., 3–9); and (iii) Cultural services (i.e., 10–12). The qualitative ecosystem services categories offered by each habitat were based on Table 1 from Salomidi et al. (2012), which, in turn, classified them based on an adaptation of the categories proposed by the Millennium Ecosystem Assessment (MEA, 2003) and Beaumont et al. (2007). Rather than using absolute metrics to classify services of each habitat, the assessment was based on the expert judgment of Salomidi et al. (2012), collated in the aforementioned **Table 1** of that manuscript, and the following guidelines: (i) when the provision of a specific service is well documented in the scientific literature and is widely accepted as important for the specific benthic habitat analyzed, it was considered as providing a "High" value for such ecosystem service (e.g., the role of seagrass beds in sediment retention and prevention of coastal erosion); (ii) when a service was or could be provided by a habitat but to a substantially lower magnitude than by other habitats and without being vital for the persistence of an important human activity, a "Low" value was assigned; and (iii) in all other cases, ecosystem services were classified as "Negligible/Irrelevant/Unknown." For the purpose of the present investigation, ecosystem services categories were rated into the following numerical values for further analysis: "High = 3," "Low = 1," "Negligible/Irrelevant/Unknown = 0." A similar classification and scores were successfully used in smaller areas (Potts et al., 2014) (see **Figures 3**, **4** in that manuscript).

The ecosystem services provisioning categories of each habitat type, was linked to the final habitat map. For those habitat classes that were included in the map, but not listed in Salomidi et al. (2012), the categories were assigned according to the knowledge of the authors, in a similar way to that of Potts et al. (2014).

To analyse the spatial distribution pattern of ecosystem services provisioning levels, the total area and its percentage cover of the total mapped area, mean depth, and mean distance to the coastline were calculated. The values of all cells encompassed within a polygon representing the extent of a habitat, were averaged to assign a unique value to each polygon for each variable (i.e., mean depth value within a polygon) To assess whether the distance to the coastline and depth had an effect on the categories at which the different ecosystem services are provided (i.e., high, low, and negligible values), Kruskal-Wallis non-parametric tests were applied using Statgraphics v.5.0. Then, differences in ecosystem services categories within the subregions were tested using Chi-Square tests. Finally, Friedman test, followed by *post-hoc* Wilcoxon tests, was undertaken to explore statistical differences between ecosystem services typologies (i.e., provision, regulation, and cultural).

RESULTS

The European North Atlantic Ocean (EEZ only) covers more than 4.5 million km^2 (**Table 1**), of which 26% corresponds to continental shelf (up to 200 m depth) and 74% to deeper areas (**Figure 2**). To date, 88% of the continental shelf and 18% of the deeper areas have been mapped, accounting for 38.9 % of the total EEZ area of the European North Atlantic Ocean.

The Macaronesia accounts for the highest proportion of the European North Atlantic EEZ, followed by the Extended North Sea (**Table 1**). However, differences in the amount of mapped area can be found among sub-regions. Whereas countries located in the Celtic Sea and North Sea have already mapped almost all their EEZ seabed surface (i.e., 98 and 93%, respectively), countries located in Macaronesia, Bay of Biscay, and Iberian coasts (i.e., France, Portugal, and Spain) have still more than 80% of the seabed area without cartographic information (**Table 1** and

Table 1 | Total spatial contribution of each sub-region to the Exclusive Economic Zone (EEZ) of the European North Atlantic Ocean, and their mapped area, represented in total and relative (%) terms.

Subregion	EEZ of the European North Atlantic Ocean		Mapped area of the EEZ of the European Atlantic Ocean	
	Total area (km^2)	Total area (%)	Total mapped area (km^2)	Total mapped area (%)
Macaronesia	2,119,095	47	88,150	4
Bay of Biscay and Iberian peninsula	818,491	18	154,472	19
Celtic Sea	550,606	12	541,042	98
Extended North Sea	1,051,611	23	981,633	93
TOTAL	4,539,803	100	1,765,297	39

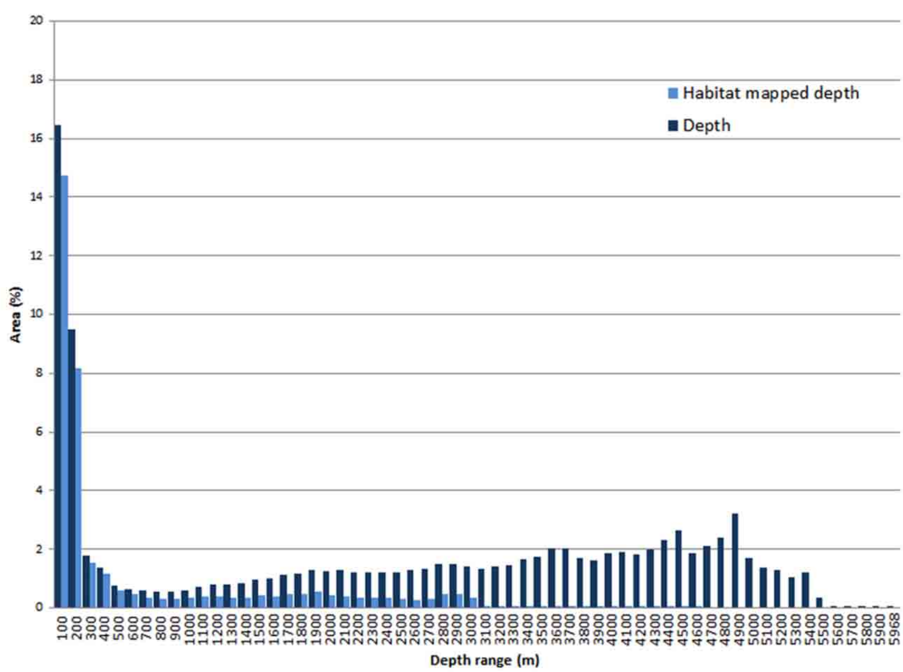

FIGURE 2 | Depth distribution of the Exclusive Economic Zone of the European North Atlantic Ocean (dark blue) and depth distribution of habitat-mapped areas (light blue).

Figure S1). Indeed, habitat maps for the Canary and Madeira Archipelagos, in Macaronesia, are not available. It should be highlighted that these countries have some of the most extensive and deepest EEZs areas of the European North Atlantic Ocean.

The 1.7 million km^2 covered by the integrated broad-scale habitat map encompassed 62 different benthic habitats and seabed seascape features (**Figure 3**). The North Sea and the Celtic Sea encompassed 58 and 55 habitats respectively, while the Bay of Biscay and Macaronesia only covered 42 and 20 habitats, respectively. Furthermore, very few habitats accounted for a large section of the mapped area (**Figure 4**). Ten habitats covered more than 75% of the total mapped area, of which deep sea mud (18.3%), deep circalittoral sand (16.2%), circalittoral fine sands, or circalittoral muddy sand (9.7%) were the most dominant ones. Opposite, a large number of habitats (i.e., 33) covered less than 10,000 km^2 or 0.5% of the mapped seabed. The least dominant habitats in the European North Atlantic Ocean were the low energy infralittoral mixed hard sediments, Atlantic and Mediterranean low energy infralittoral rock and sponge communities on deep circalittoral rock, all of which cover less than 100 km^2.

Of the 62 habitats identified in European North Atlantic Ocean, none of them provides the 12 ecosystem services considered in this study at the highest value (**Table 2**). However, four of these habitats (i.e., Infralittoral rock and other hard substrata, Atlantic and Mediterranean high energy infralittoral rock, High energy infralittoral seabed, and High energy infralittoral mixed hard sediments) provide high values for 11 services (excluding nutrient cycling). Another seven infralittoral habitats also provide high values for 10 of the services. On the other hand, 12 deep and bathyal habitats are considered as providing negligible values for 10 or more ecosystem services. The upper, mid, and lower bathyal seabed habitats provide the lowest number of ecosystem services and values.

Results also indicate that the highest provision of services is that of habitats located close to the coastline and in shallow waters ($p < 0.001$ for all services and in both cases—distance and depth; see **Tables 3**, **4**). Thus, there is a gradient on the level of services provision, from high to lower or negligible values, seawards and toward deeper areas. For example, areas providing high food provision services are located close to the coast (16 ± 35 km) and in shallow areas (47 ± 50 m). Furthermore, it is also observed that the level of service provision significantly varies across subregions (Chi-Square test: p always < 0.001), with the North Sea being the region generally providing services at the highest levels.

Table 2 also suggests that none of the ecosystem services is provided by all the habitats. "Food," "biodiversity maintenance" and "nursery grounds" (i.e., "reproduction") are the ecosystem services most commonly provided by habitats (and to the highest level). Opposite, "photosynthesis," "disturbance prevention," "air quality" and "cultural services" are provided on a high level by a limited number of habitats. This pattern is also observed when considering not only the number of habitats providing specific ecosystem services, but also the area providing such

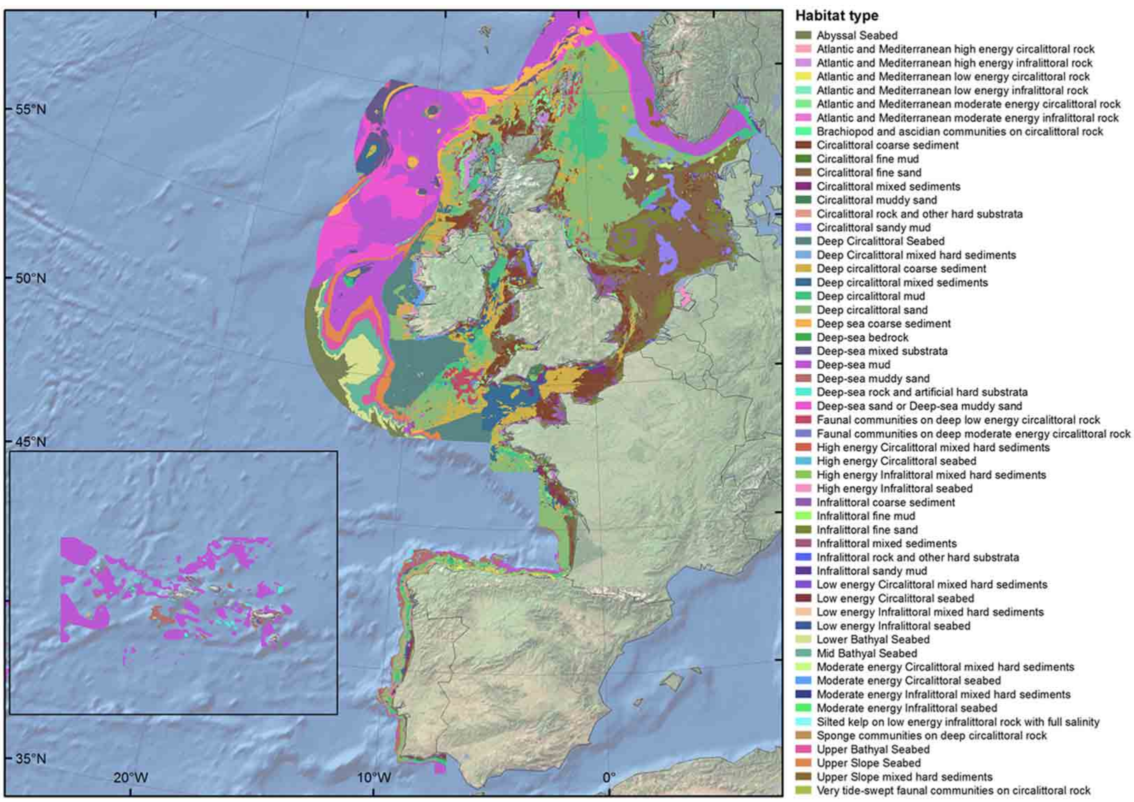

FIGURE 3 | Benthic habitat map distribution within the European North Atlantic Ocean. Habitats are listed in alphabetical order.

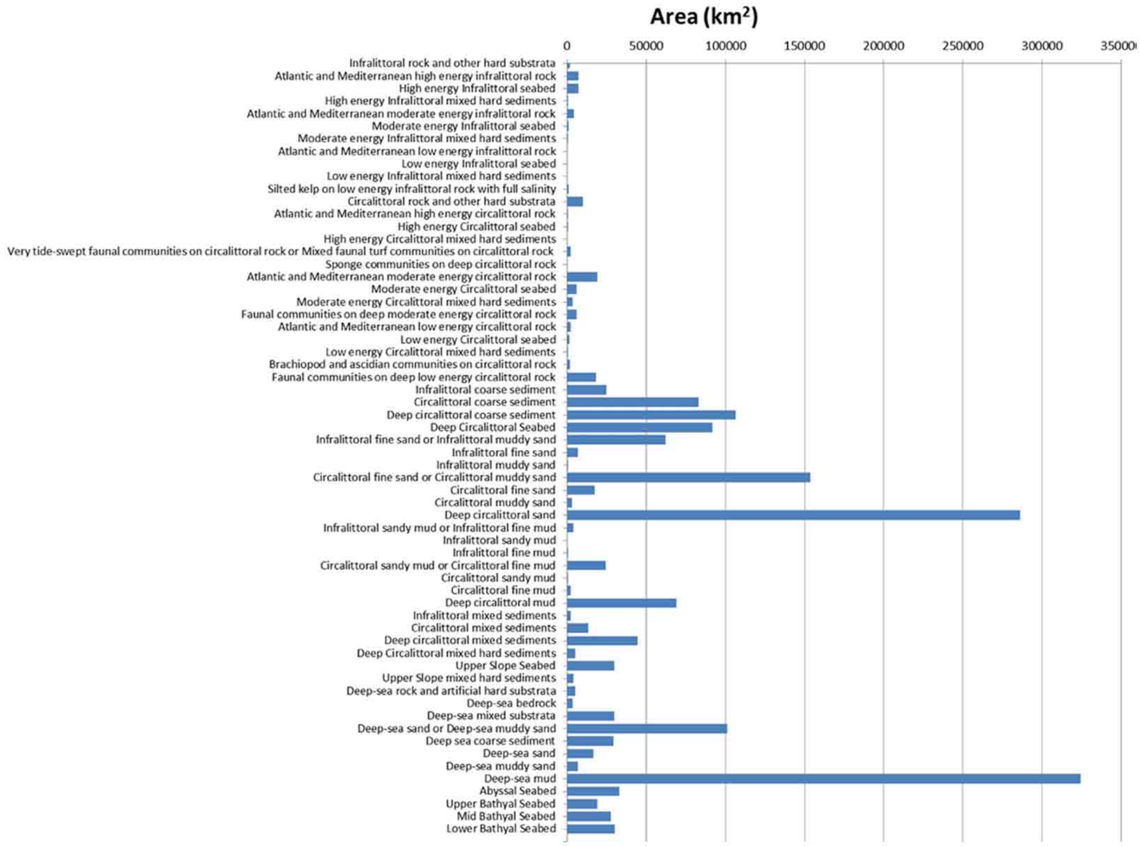

FIGURE 4 | Area covered by each benthic habitat and seascape feature type, within the European North Atlantic Ocean.

ecosystem services (**Table 3** and **Figures S2–S13**, in Supporting Information).

Indeed, 93% of the studied area provides food provision services, of which 62% corresponds with high food provision values. Similarly, a high proportion of the mapped area (99%) is considered as providing high (41%) and low (58%) biodiversity maintenance services.

The next ecosystem services, in terms of area coverage, are reproduction and nursery, which are provided by 53% of the mapped area. For the remaining ecosystem goods and services (i.e., air quality and climate regulation, water quality regulation and bioremediation, nutrient cycling, raw material provision, photosynthesis, chemosynthesis, and primary production), the area covered by habitats providing them at high values is much smaller. The disturbance and natural hazard prevention service has the smallest spatial coverage.

Finally, cultural services (i.e., cognitive value, leisure, recreation and cultural inspiration, and feel good and warm glow), showed similar patterns on their spatial distribution. The area covered by the habitats providing such type of services (both, at high and low levels) is very limited (around 11% of the total).

On the other hand, significant differences are observed in the spatial distribution of provision levels of aggregated ecosystem services (i.e., provisioning, regulating, and cultural), (Friedman test $\chi^2 = 47,858$; $p < 0.001$) (**Figure 5**). The provisioning services are supplied at significantly higher levels than both regulating (Wilcoxon *post-hoc* test $z = -154$, $p < 0.001$) and cultural services (Wilcoxon *post-hoc* test $z = -171$, $p < 0.001$); and in turn, regulating services are also provided at significantly higher levels than cultural services (Wilcoxon *post-hoc* test $z = -130$, $p < 0.001$).

DISCUSSION

Seafloor maps are an essential source of information for resource exploitation and management purposes (Rice, 2010). Nevertheless, in Europe, it is worth noting that countries such as Spain, Portugal and France, with large EEZ areas have less mapped areas. This is probably due to the steepness of the seafloor, with large bathyal and abyssal areas, and the technical and economic challenge associated with mapping areas with such characteristics. Among others, marine shallow water areas support most of the human activities associated with the use and benefit of the ecosystem services provided by benthic habitats (Ramirez-Llodra et al., 2011; Korpinen et al., 2013), but accurate estimation of the values of services and their spatial distribution is not available for extensive areas. Within this research, the assessment and mapping of the ecosystem services provided by benthic habitats of the European North Atlantic Ocean has been undertaken for the first time.

Table 2 | Ecosystem services assessment for each habitat and seabed feature type (H, high; L, low; and N, Negligible).

Habitat name	EUNIS code	Food	Raw material	Air quality	Disturbance	Photosynthesis	Nutrient	Reproduction	Biodiversity	Wate	Cognitive	Leisure	Feelgood
Infralittoral rock and other hard substrata	A3*	H	H	H	H	H	L	H	H	H	H	H	H
Atlantic and Mediterranean high energy infralittoral rock	A3.1*	H	H	H	H	H	L	H	H	H	H	H	H
High energy infralittoral seabed		H	H	H	H	H	L	H	H	H	H	H	H
High energy infralittoral mixed hard sediments		H	H	H	H	H	L	H	H	H	H	H	H
Atlantic and Mediterranean moderate energy infralittoral rock	A3.2*	H	H	H	L	H	H	H	H	H	H	H	L
Moderate energy infralittoral seabed		H	H	H	L	H	H	H	H	H	H	H	L
Moderate energy infralittoral mixed hard sediments		H	H	H	L	H	H	H	H	H	H	H	L
Atlantic and Mediterranean low energy infralittoral rock	A3.3*	H	H	H	L	H	H	H	H	H	H	H	L
Low energy infralittoral seabed		H	H	H	N	H	H	H	H	H	H	H	L
Low energy infralittoral mixed hard sediments		H	H	H	N	H	H	H	H	H	H	H	L
Silted kelp on low energy infralittoral rock with full salinity	A3.31	H	H	H	N	H	H	H	H	H	H	H	L
Circalittoral rock and other hard substrata	A4*	H	H	L	H	N	H	H	H	H	H	L	L
Atlantic and Mediterranean high energy circalittoral rock	A4.1*	H	H	L	H	N	H	H	H	H	H	L	L
High energy circalittoral seabed		H	H	L	H	N	H	H	H	H	H	L	L
High energy circalittoral mixed hard sediments		H	H	L	H	N	H	H	H	H	H	L	L
Very tide-swept faunal communities on circalittoral rock or mixed faunal turf communities on circalittoral rock	A4.11 or A4.13*	H	H	N	H	N	H	H	H	H	L	L	L
Sponge communities on deep circalittoral rock	A4.12	H	H	N	H	N	H	H	H	H	H	L	L
Atlantic and Mediterranean moderate energy circalittoral rock	A4.2*	L	L	L	N	N	H	H	H	H	H	L	L
Moderate energy circalittoral seabed		L	N	L	N	N	H	H	H	H	H	L	L
Moderate energy circalittoral mixed hard sediments		L	N	L	N	N	H	H	H	H	H	L	L
Faunal communities on deep moderate energy circalittoral rock	A4.27	L	L	L	N	L	H	H	H	H	H	L	L
Atlantic and Mediterranean low energy circalittoral rock	A4.3*	H	L	H	N	L	H	H	H	H	H	H	L
Low energy circalittoral seabed		H	L	L	N	N	H	H	H	H	H	H	L
Low energy circalittoral mixed hard sediments		H	L	L	N	N	H	H	H	H	H	H	L
Brachiopod and ascidian communities on circalittoral rock	A4.31	L	L	L	L	L	L	L	H	L	H	H	L
Faunal communities on deep low energy circalittoral rock	A4.33	H	L	H	N	L	H	H	H	H	H	H	H
Infralittoral coarse sediment	A5.13*	H	H	N	N	N	L	H	N	N	N	L	L
Circalittoral coarse sediment	A5.14*	H	H	N	N	N	L	L	L	N	N	N	N
Deep circalittoral coarse sediment	A5.15*	H	L	N	N	N	L	N	L	N	N	N	N
Deep circalittoral seabed		H	L	N	N	N	L	N	L	N	N	N	N
Infralittoral fine sand or infralittoral muddy sand	A5.23* or A5.24*	H	L	N	N	N	L	H	L	N	N	L	L
Infralittoral fine sand	A5.23*	H	L	N	N	N	L	H	L	N	N	L	L
Infralittoral muddy sand	A5.24*	H	L	N	N	N	L	H	L	N	N	L	L
Circalittoral fine sand or circalittoral muddy sand	A5.25* or A5.26*	H	L	N	N	N	L	H	L	N	N	N	N
Circalittoral fine sand	A5.25*	H	L	N	N	N	L	H	L	N	N	N	N
Circalittoral muddy sand	A5.26*	H	L	N	N	N	L	L	L	L	N	N	N
Deep circalittoral sand	A5.27	H	L	N	L	N	L	L	L	L	N	N	N

(Continued)

Table 2 | Continued

Habitat name	EUNIS code	Food	Raw material	Air quality	Disturbance	Photosynthesis	Nutrient	Reproduction	Biodiversity	Wate	Cognitive	Leisure	Feelgood
Infralittoral sandy mud or infralittoral fine mud	A5.33* or A5.34*	H	N	N	N	N	L	L	L	L	N	N	N
Infralittoral sandy mud	A5.33*	H	N	N	N	N	L	L	L	L	N	N	N
Infralittoral fine mud	A5.34*	L	N	N	N	N	L	N	L	L	N	N	N
Circalittoral sandy mud or circalittoral fine mud	A5.35* or A5.36*	H	N	N	N	N	L	L	L	L	N	N	N
Circalittoral sandy mud	A5.35*	H	N	N	N	N	L	L	L	L	N	N	N
Circalittoral fine mud	A5.36*	H	N	N	N	N	L	L	L	L	N	N	N
Deep circalittoral mud	A5.37*	H	N	N	N	N	L	L	L	L	N	N	N
Infralittoral mixed sediments	A5.43*	H	L	N	N	N	L	L	H	L	N	N	N
Circalittoral mixed sediments	A5.44*	H	L	N	N	N	L	L	H	L	N	N	N
Deep circalittoral mixed sediments	A5.45*	H	L	N	N	N	L	L	H	L	N	N	N
Deep circalittoral mixed hard sediments		H	N	N	N	N	N	H	H	N	N	N	N
Upper slope seabed		H	N	N	N	N	N	L	H	N	N	N	N
Upper slope mixed hard sediments		H	N	N	N	N	N	L	H	N	N	N	N
Deep-sea rock and artificial hard substrata	A6.1*	L	N	N	N	N	N	N	H	N	N	N	N
Deep-sea bedrock	A6.11	N	N	N	N	N	N	N	H	N	N	N	N
Deep-sea mixed substrata	A6.2	L	N	N	N	N	N	N	H	N	N	N	N
Deep-sea sand or deep-sea muddy sand	A6.3* or A6.4	L	N	N	N	N	N	N	H	N	N	N	N
Deep sea coarse sediment		L	N	N	N	N	N	N	H	N	N	N	N
Deep-sea sand	A6.3*	L	N	N	N	N	N	N	H	N	N	N	N
Deep-sea muddy sand	A6.4	L	N	N	N	N	N	N	H	N	N	N	N
Deep-sea mud	A6.5	L	N	N	N	N	N	N	H	N	N	N	N
Abyssal seabed		N	N	N	N	N	N	N	L	N	H	N	N
Upper bathyal seabed		N	N	N	N	N	N	N	L	N	L	N	N
Mid bathyal seabed		N	N	N	N	N	N	N	L	N	L	N	N
Lower bathyal seabed		N	N	N	N	N	N	N	L	N	L	N	N

*EUNIS habitat code is given for those habitats included in the classification; * indicates that the assessment was based upon Salomidi et al. (2012).*

In the studied area, a clear gradient has been identified for the provision of ecosystem services, with significantly higher provision levels for habitats located in shallow waters and close to the shore. This is coherent with the fact that habitats provide more ecosystem services as people have easier access to them. In fact, accessibility is a crucial factor and it is typically included in the monetization of some services, especially for cultural services (Milcu et al., 2013). In the case of benthic habitats, access depends on depth, and generally, on the distance from the coastline. Therefore, deep-sea habitats and habitats located further away from the coast generally provide fewer ecosystem services and at lower degree due to limited access and lack of scientific knowledge for most of them. However, as exploration of the deep-sea improves with recent technological advances, access to such habitats (Ramirez-Llodra et al., 2011) will become less difficult, increasing the ecosystem services that they provide in the near future (Thurber et al., 2013).

According to our estimations, between 93 and 99% (depending on the sub-regions) of the benthic habitats of the European North Atlantic Ocean deliver food provision and biodiversity maintenance services; meanwhile, reproduction and nursery services are provided by 53% of the area. We consider that the assessment of this last service could be underestimated due the fact that knowledge on life-cycles is mainly limited to commercially important species. But it should be taken into account that other non-commercial species, with unknown life cycles, also play an important role in food webs. Thus, the reproduction and nursery grounds are likely to cover a wider area than the one resulting from this investigation. In contrast, areas providing other services are smaller or have much more limited spatial distribution. For example, the area corresponding to habitats that supply raw materials is very limited, and the highest proportion of this area only provides low or negligible resources. To explain this pattern, it should be considered that few raw materials are exploited at present, and that their exploitation is regulated by national and international regulations as the impacts associated with such exploitation may be high. However, there may be high potential for habitats to provide higher provision of this service as new raw materials are discovered and exploited (i.e., pharmaceutical).

Another interesting pattern is that observed for the provision of coastal protection as an ecosystem service. Liquete et al. (2013b) propose the use of 14 biophysical and socio-economic

Table 3 | Depth, distance to the coast, and area covered by the ecosystem services assigned with different assessment categories (i.e., High, Low, and Negligible) and provided by benthic habitats, within the Atlantic Ocean, and for each of the sub-regions.

Ecosystem service	Categories	Macaronesia				Bay of Biscay				Celtic Sea				North Sea				Total			
		Area (km^2)	Area (%)	Depth (m)	Distance (km)	Area (km^2)	Area (%)	Depth (m)	Distance (km)	Area (km^2)	Area (%)	Depth (m)	Distance (km)	Area (km^2)	Area (%)	Depth (m)	Distance (km)	Area (km^2)	Area (%)	Mean depth (m) ± *SD*	Mean distance (km) ± *SD*
Food provision	High	1421	2	97 ± 82	3 ± 7	120811	78	37 ± 42	7 ± 12	278777	52	42 ± 47	18 ± 39	699171	71	37 ± 48	15 ± 35	1101365	62	47 ± 50	16 ± 35
	Low	86742	98	983 ± 919	40 ± 71	33450	22	191 ± 268	17 ± 18	153104	28	88 ± 208	52 ± 95	277165	28	91 ± 203	15 ± 44	550790	31	186 ± 397	24 ± 56
	Negligible	0	0	0	0	95	0	1091 ± 254	247 ± 2	109114	20	1116 ± 799	230 ± 112	4583	0	730 ± 535	152 ± 129	113787	6	917 ± 579	193 ± 122
Raw materials (biological) (incl. biochemical medicinal and ornamental)	High	662	1	78 ± 82	2 ± 5	13767	9	27 ± 33	4 ± 7	37213	7	26 ± 27	6 ± 20	95802	10	25 ± 29	8 ± 22	148244	8	33 ± 33	8 ± 22
	Low	759	1	111 ± 78	3 ± 8	100032	65	43 ± 40	8 ± 12	198619	37	53 ± 42	25 ± 43	541029	55	48 ± 52	21 ± 40	840706	48	57 ± 50	21 ± 39
	Negligible	86742	98	981 ± 919	40 ± 71	40556	26	195 ± 273	18 ± 25	305162	56	187 ± 428	69 ± 108	344088	35	145 ± 308	27 ± 69	776992	44	240 ± 455	37 ± 78
Air quality and climate regulation	High	267	<1	55 ± 79	1 ± 3	3939	3	24 ± 32	4 ± 8	13809	3	18 ± 23	6 ± 23	27256	3	29 ± 37	4 ± 17	45857	3	34 ± 38	5 ± 19
	Low	238	<1	107 ± 94	3 ± 6	10830	7	49 ± 46	7 ± 9	13329	2	43 ± 34	16 ± 39	26609	3	49 ± 49	6 ± 19	51251	3	59 ± 47	9 ± 25
	Negligible	87658	99	457 ± 724	18 ± 49	139586	90	68 ± 142	9 ± 16	513858	95	71 ± 189	29 ± 60	927054	95	63 ± 164	26 ± 50	1668835	95	91 ± 237	26 ± 51
Disturbance and natural hazard prevention	High	506	1	77 ± 89	2 ± 4	7655	5	34 ± 42	4 ± 7	9873	2	24 ± 27	3 ± 8	13447	1	18 ± 24	2 ± 5	32008	2	31 ± 36	3 ± 7
	Low	59	0	164 ± 58	7 ± 17	46154	30	47 ± 46	8 ± 11	44343	8	59 ± 44	26 ± 34	204484	21	40 ± 39	11 ± 26	295141	17	53 ± 42	15 ± 29
	Negligible	87599	99	474 ± 741	19 ± 50	100546	65	67 ± 141	9 ± 16	486779	90	69 ± 192	29 ± 63	762988	78	63 ± 149	21 ± 47	1438794	81	88 ± 223	23 ± 50
Photosynthesis. chemosynthesis and primary production	High	267	0	55 ± 79	1 ± 3	1710	1	11 ± 9	2 ± 3	5072	1	14 ± 17	5 ± 23	17273	2	25 ± 32	3 ± 9	24898	1	28 ± 3	4 ± 13
	Low	0	0	0	0	2229	1	76 ± 37	13 ± 14	10247	2	71 ± 36	22 ± 22	16548	2	67 ± 47	15 ± 37	29053	2	77 ± 40	17 ± 37
	Negligible	87896	100	415 ± 690	16 ± 46	150417	97	64 ± 129	9 ± 15	525676	97	65 ± 169	26 ± 56	947098	97	59 ± 145	20 ± 45	1711992	97	83 ± 211	22 ± 47
Nutrient cycling	High	238	<1	107 ± 94	3 ± 6	13537	9	47 ± 45	7 ± 10	23753	4	39 ± 33	15 ± 37	42393	4	44 ± 47	5 ± 20	80372	5	53 ± 45	9 ± 25
	Low	1183	1	95 ± 79	3 ± 7	111720	72	34 ± 38	7 ± 11	236946	44	42 ± 41	18 ± 36	668826	68	35 ± 43	19 ± 38	1019454	58	44 ± 45	18 ± 36
	Negligible	86742	98	983 ± 919	40 ± 71	29099	19	407 ± 310	33 ± 30	280296	52	627 ± 691	224 ± 105	269700	27	524 ± 499	101 ± 113	666116	38	642 ± 633	94 ± 110
Reproduction and nursery	High	795	1	73 ± 80	2 ± 6	27547	18	29 ± 34	5 ± 8	48651	9	28 ± 29	11 ± 31	291708	30	33 ± 39	13 ± 33	369716	21	39 ± 39	13 ± 33
	Low	566	1	121 ± 75	3 ± 8	78075	51	49 ± 49	10 ± 15	127503	24	59 ± 60	26 ± 41	364004	37	46 ± 51	17 ± 35	570291	32	59 ± 55	19 ± 36
	Negligible	86802	98	893 ± 905	36 ± 68	48733	32	229 ± 283	22 ± 23	364841	67	194 ± 415	75 ± 105	325207	33	230 ± 372	45 ± 82	825936	47	314 ± 499	51 ± 84
Maintenance of biodiversity	High	87641	99	456 ± 733	18 ± 49	58162	38	95 ± 176	11 ± 17	213522	39	50 ± 114	24 ± 60	355537	36	61 ± 146	11 ± 38	716000	41	95 ± 236	16 ± 45
	Low	475	1	103 ± 73	4 ± 10	95430	62	36 ± 43	7 ± 12	323645	60	68 ± 191	23 ± 46	605026	62	45 ± 100	23 ± 42	1024886	58	55 ± 122	23 ± 42
	Negligible	48	0	54 ± 26	4 ± 9	763	0	12 ± 9	2 ± 2	3828	1	18 ± 16	6 ± 17	20355	2	20 ± 17	21 ± 42	25057	1	21 ± 16	17 ± 38
Water quality regulation and bioremediation of waste	High	506	<1	77 ± 89	2 ± 4	14769	10	39 ± 42	6 ± 9	28149	5	30 ± 31	11 ± 32	53342	5	38 ± 44	5 ± 18	97600	6	46 ± 45	7 ± 22
	Low	458	<1	125 ± 78	3 ± 8	73241	47	51 ± 48	10 ± 13	78298	14	58 ± 46	26 ± 36	301596	31	43 ± 44	16 ± 35	453678	26	58 ± 49	18 ± 34
	Negligible	87199	99	618 ± 835	25 ± 58	66345	43	78 ± 175	9 ± 17	434548	80	82 ± 242	32 ± 72	625980	64	72 ± 194	30 ± 55	1214665	69	108 ± 287	30 ± 59
Cognitive value	High	506	1	77 ± 89	2 ± 4	14769	10	39 ± 42	6 ± 9	60097	11	36 ± 131	12 ± 35	53946	5	39 ± 45	5 ± 18	130136	7	48 ± 72	7 ± 23
	Low	0	0	0	0	95	0	445 ± 603	99 ± 135	77252	14	393 ± 688	62 ± 113	2371	0	130 ± 356	15 ± 44	79733	5	202 ± 469	28 ± 71
	Negligible	87658	99	457 ± 724	18 ± 49	139491	90	68 ± 141	9 ± 15	403646	75	58 ± 101	28 ± 56	924601	94	60 ± 150	26 ± 51	1556074	88	87 ± 222	26 ± 50
Leisure, recreation and cultural inspiration	High	267	<1	55 ± 79	1 ± 3	3939	3	24 ± 32	4 ± 8	14603	3	24 ± 29	13 ± 37	30729	3	39 ± 47	4 ± 17	50162	3	44 ± 47	7 ± 23
	Low	411	<1	80 ± 81	2 ± 5	13948	9	31 ± 39	5 ± 7	23697	4	29 ± 29	7 ± 23	106159	11	30 ± 32	14 ± 36	144690	8	37 ± 35	14 ± 33
	Negligible	87485	99	514 ± 756	20 ± 52	136468	88	83 ± 157	11 ± 17	502696	93	85 ± 209	35 ± 65	844031	86	82 ± 194	27 ± 53	1571091	89	114 ± 270	29 ± 54
Feel good or warm glow	High	267	<1	55 ± 79	1 ± 3	1233	1	10 ± 9	1 ± 2	12957	2	16 ± 23	3 ± 12	20931	2	26 ± 38	5 ± 22	35761	2	30 ± 40	5 ± 19
	Low	411	<1	80 ± 81	2 ± 5	16654	11	33 ± 39	5 ± 8	25343	5	31 ± 30	12 ± 33	115957	12	36 ± 40	11 ± 30	159090	9	42 ± 41	11 ± 30
	Negligible	87485	99	514 ± 756	20 ± 52	136468	88	83 ± 157	11 ± 17	502696	93	85 ± 209	35 ± 65	844031	86	82 ± 194	27 ± 53	1571091	89	114 ± 270	29 ± 52

Table 4 | Differences (Kruskal-Wallis test) between ecosystem services categories provided by benthic habitats, according to the distance to coastline, and depth ($N = 55,023$).

Ecosystem service	Distance to coastline			Depth		
	Category	Kruskal-Wallis (H)	*p*	Category	Kruskal-Wallis (H)	*p*
Food provision	High[a] Low[b] Negligible[c]	1024.4	<0.001***	High[a] Low[b] Negligible[c]	4181.0	<0.001***
Raw materials (biological) (incl. Biochemical. medicinal and ornamental)	High[a] Low[b] Negligible[c]	4842.1	<0.001***	High[a] Low[b] Negligible[c]	5531.1	<0.001***
Air quality and climate regulation	High[a] Low[b] Negligible[c]	8416.0	<0.001***	High[a] Low[b] Negligible[c]	2676.8	<0.001***
Disturbance and natural hazard prevention	High[a] Low[b] Negligible[c]	5595.6	<0.001***	High[a] Low[b] Negligible[c]	2799.6	<0.001***
Photosynthesis, chemosynthesis and primary production	High[a] Low[b] Negligible[c]	6354.9	<0.001***	High[a] Low[b] Negligible[b]	4426.9	<0.001***
Nutrient cycling	High[a] Low[b] Negligible[c]	5288.0	<0.001***	High[a] Low[b] Negligible[c]	7653.9	<0.001***
Reproduction and nursery	High[a] Low[b] Negligible[c]	4543.1	<0.001***	High[a] Low[b] Negligible[c]	8444.5	<0.001***
Maintenance of biodiversity	High[a] Low[b] Negligible[a]	3786.5	<0.001***	High[a] Low[b] Negligible[b]	1617.1	<0.001***
Water quality regulation and bioremediation of waste	High[a] Low[b] Negligible[c]	8391.6	<0.001***	High[a] Low[b] Negligible[c]	548.9	<0.001***
Cognitive value	High[a] Low[b] Negligible[b]	8252.1	<0.001***	High[a] Low[b] Negligible[c]	202.0	<0.001***
Leisure, recreation and cultural inspiration	High[a] Low[b] Negligible[c]	8687.9	<0.001***	High[a] Low[b] Negligible[c]	4065.5	<0.001***
Feel good or warm glow	High[a] Low[b] Negligible[c]	8105.2	<0.001***	High[a] Low[b] Negligible[c]	4785.2	<0.001***

****Indicates significant results at 0.001 significance level. The superscripts within each service have been used to indicate significant (different superscripts) or non-significant (equal superscripts) differences on post-hoc tests between pairs of data, at 0.05 significance level.*

variables, from both terrestrial and marine datasets, in assessing coastal protection. In this investigation, we have only used benthic habitats, which may explain the relatively small area providing this service in the European North Atlantic Ocean. Furthermore, it is the limited distribution of biogenic structures and seagrass species within this ocean, considered as the main producer of this service, which may explain the limited provision to shallow and habitats located close to the

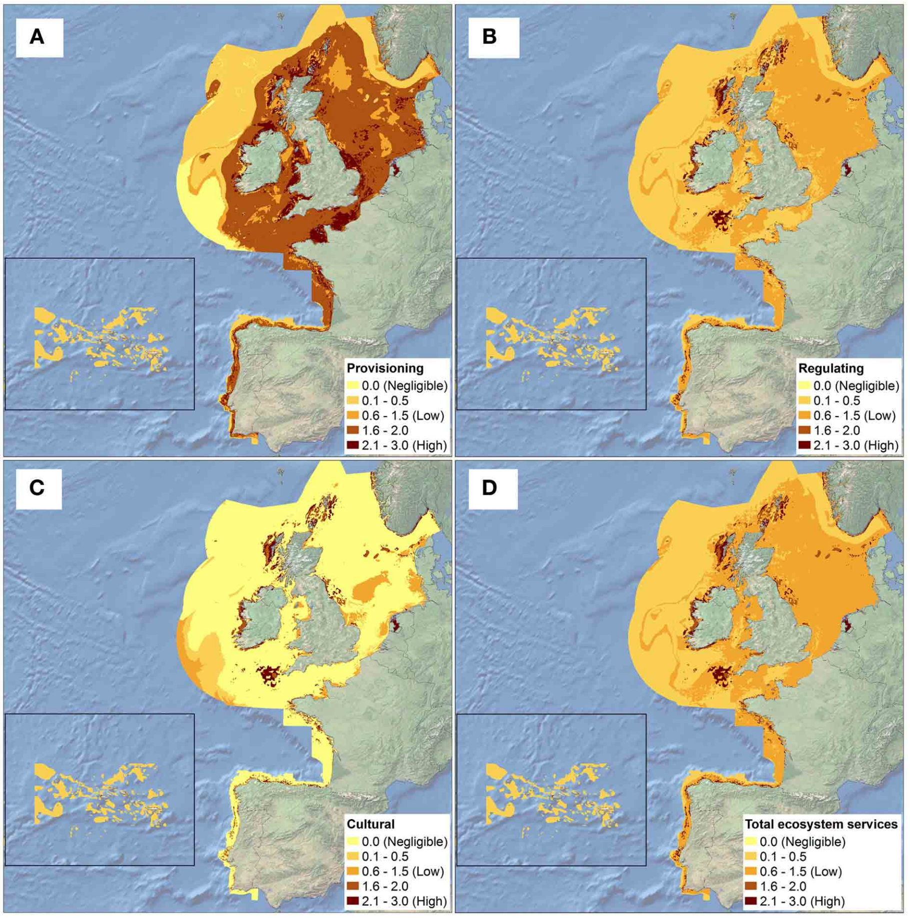

FIGURE 5 | Spatial distribution of the mean value of aggregated ecosystem: (A) Provisioning services; (B) Regulating services; (C) Cultural services; and (D) Total ecosystem services.

coast (Christianen et al., 2013; Cullen-Unsworth and Unsworth, 2013).

The remaining ecosystem services are provided in limited areas. This pattern is possibly explained by the fact that some of the services analyzed are provided by very specific, spatially limited benthic habitats (i.e., photic zones), or in a larger scale, by pelagic habitats, i.e., air quality and climate regulation, water quality regulation and bioremediation, nutrient cycling, photosynthesis, chemosynthesis, and primary production. For example, some of them, such as climate regulation or carbon sequestration, are very important in coastal margin habitats, rather than in subtidal habitats (Beaumont et al., 2014).

Very small areas (11%) have been identified as providing cultural services (i.e., cognitive, leisure, recreation and cultural inspiration, feel good, and warm glow). This result is likely to be a consequence of the dependence of these services on accessibility. Therefore, even if the current provision of these services is limited to few habitats and areas (which are probably heavily used), it is likely that over time, as access increases to certain areas, these services will increase their value and distribution (Ghermandi et al., 2012). The broad-scale spatial patterns of the ecosystem services assessment resulting from this investigation could be considered consistent for different spatial scales of analysis if the approach is implemented elsewhere.

When considering the approach and results obtained through this research, authors would like to highlight that, rather than getting a valuation of the ecosystem services provided by the benthic habitats of the European North Atlantic Ocean, in our investigation a pragmatic approach for benthic services mapping is applied, based on the best available knowledge (De Groot et al., 2010). We recognize that the reliability of the results obtained in this investigation depend on, among other things, two major aspects: (i) the quality and reliability of benthic habitat maps used, which is an important but insufficiently assessed issue (Schägner et al., 2013); and (ii) the valuation of the ecosystem services carried out by scientific expert judgment (extracted from Salomidi et al., 2012), which could be biased toward the knowledge of the experts who published that research; meanwhile, social and economic aspects could be under-rated.

Some of the aforementioned weaknesses could be overcome: (i) enhancing the scientific knowledge of marine ecosystem functioning by finalizing detailed benthic habitat maps of the complete study area (especially, for the EEZ of France, Spain, and Portugal and deeper benthic habitats; Liquete et al., 2013a); and (ii) improving the assessment of services valuation, promoting the multidisciplinary discussions among environmental and social scientists and economists, to achieve consensus on benthic habitat services values.

A more adequate ecosystem services assessment and valuation could be carried out following the steps below:

(i) Definition of marine ecosystem services categories, based upon those already in use (see Liquete et al., 2013a). This definition should be carried out by experts from different scientific disciplines such as environmental, social (including stakeholders' participation) and economical sciences. In order to ensure consistency and allow for aggregation or comparison of results across the countries, there is a need for a common classification and to define which ecosystems and services will be considered as a priority by Member States (Maes et al., 2013).
(ii) Mapping services based on spatial distribution and patterns of different ecosystem components, processes and their relationships, including the need for future scenarios.
(iii) Biological and environmental valuation services by common procedures, undertaken by environmental, social, and economic scientists. Many ecosystem services cannot be directly quantified and thus, researchers must rely on indicators or proxy data for their quantification (Liquete et al., 2013a). Expert judgment may be a very important source of information, but the careful selection of a broad panel of experts may be required for ecosystem service assessment.
(iv) Economic valuation undertaken by economists and social scientists. No single ecological, social or economic methodology can capture the total value of these complex systems (Wilson et al., 2013). Assigning economic values to seascape features and habitat functions of marine ecosystems requires full understanding of the natural systems upon which they rely (Wilson et al., 2013). Probably, new economic valuation methods should be adopted (see Liquete et al., 2013a).
(v) Ecosystem services valuation assessment, which could assist in the determination of the ecological and environmental status under the Water Framework Directive (WFD) and MSFD, respectively (Katsanevakis et al., 2011; Vlachopoulou et al., 2014).

This process could result in the definition of proposals for management plans for different directives (e.g., MSFD, Habitats Directive) and instruments such as Marine Spatial Planning. Since oceans are facing an increasing number of human uses and threats, the inclusion of ecosystem services within management plans is growing in importance. In this context, the science of ecology must play a crucial role in bringing concepts like ecosystem goods and services to the forefront of the valuation debate (Bingham et al., 1995; Wilson and Carpenter, 1999; Liquete et al., 2013a).

The spatially explicit nature of the approach presented in this investigation is of special interest to support decision-making approaches and different aspects of the ecosystem-based marine spatial management *sensu* Katsanevakis et al. (2011). Among other things, the key to achieving a more comprehensive set of management mechanisms is, in the first instance, to know more about the ecosystem functions of benthic habitats (Martinez et al., 2011). In this way, there is a key goal of maintaining the delivery of ecosystem services, which must be based upon ecological principles that articulate the scientifically-recognized attributes of healthy functioning ecosystems (Foley et al., 2010), as required by the MSFD (Borja et al., 2013; Tett et al., 2013). This would require management measures for minimizing environmental impact and maximizing the socio-economic benefit of marine services (Salomidi et al., 2012); aspects that are basic to the Marine Spatial Planning.

This research has provided a first assessment of the benthic ecosystem services at Atlantic European scale, with the provision of ecosystem services maps and their general spatial distribution patterns. Related to the objectives of this research, the conclusions are: (i) benthic habitats provide a diverse set of ecosystem services, with the food provision and biodiversity maintenance services more extensively represented. In addition, other regulating and cultural services are provided in a more limited area; and (ii) the ecosystem services assessment categories are significantly related to the distance to the coast and with depth (higher near the coast and in shallow waters).

The results obtained in this investigation highlight the need for diverse, healthy and extensive benthic habitat areas to support the provision of important and valuable ecosystem services (i.e., food provisioning, disturbance prevention, nutrient cycling, etc.). Spatially explicit assessment and valuation of ecosystem services might be of crucial interest for future management measures adoption such as Marine Spatial Planning. The approach proposed here could be considered as a pragmatic way of getting a first snapshot of the distribution of ecosystem services based on the available information and we consider this as a promising starting point for further research and discussion on ecosystem services contribution of benthic habitats in Europe.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fmars.2014.00023/abstract

Figure S1 | Depth distribution of the Exclusive Economic Zone (dark blue) and depth distribution of habitat-mapped areas (light blue), in the four subregions of the European North Atlantic Ocean; (A) Macaronesia; (B) Bay of Biscay and Iberian Coast; (C) Celtic Seas; and (D) Greater North Sea, including the Kattegat, the English Channel and Norway.

Figure S2 | Spatial distribution of food provision services.

Figure S3 | Spatial distribution of raw materials (biological, incl. biochemical, medicinal, and ornamental) services.

Figure S4 | Spatial distribution of air quality and climate regulation services.

Figure S5 | Spatial distribution of disturbance and natural hazard prevention services.

Figure S6 | Spatial distribution of photosynthesis, chemosynthesis, and primary production services.

Figure S7 | Spatial distribution of nutrient cycling services.

Figure S8 | Spatial distribution of reproduction and nursery services.

Figure S9 | Spatial distribution of maintenance of biodiversity services.

Figure S10 | Spatial distribution of water quality regulation and bioremediation of waste services.

Figure S11 | Spatial distribution of cognitive value services.

Figure S12 | Spatial distribution of leisure, recreation, and cultural inspiration services.

Figure S13 | Spatial distribution of feel good or warm glow services.

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Lessons learnt

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From Science to Policy and Society: Enhancing the Effectiveness of Communication

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Dissemination is now acknowledged as an important component of the research process, in particular for European Union (EU) funded research projects. This article builds on the authors' experience during the EU project DEVOTES (DEVelopment Of innovative Tools for understanding marine biodiversity and assessing good Environmental Status) and aims to assist other scientists to develop a successful dissemination strategy to communicate project achievements. We provide a critical review of the different tools used for outreach to our target audiences, from the academia to the policy makers, and the general public, and try to assess their impact. An effective dissemination strategy and plan should have a clear objective, be designed before the start of the project, identify the target groups and define the methods or tools to be used according to target groups and objectives. The DEVOTES dissemination strategy included two complementary approaches of communication with stakeholders: (i) traditional (e.g., peer reviewed publications, stakeholders workshops, and participation in scientific conferences), and (ii) new (e.g., social networks, smartphone applications) media tools. For each dissemination approach, we defined production targets (e.g., number of articles to be published, individual visitors on the website, etc.) to be achieved by the end of the project, and impact measurements (e.g., citation indices for peer reviewed articles) to monitor the successful implementation of DEVOTES Dissemination. This allowed us to identify which tools had been more (e.g., website) or less useful and relevant (e.g., Facebook) during the project. We conclude that impact measurements cannot be easily identified for all dissemination actions. However, for those that were possible, the DEVOTES dissemination targets were successfully achieved. Overall, the use of the tools and activities outlined in this article, combined with the constant evaluation of the dissemination goals throughout the project duration and the assessment of the effectiveness of the different tools, is essential for the achievement of an effective and timely communication of research results.

Keywords: dissemination strategy, media impact, media tools, ocean literacy networking, stakeholders, training

IMPORTANCE OF DISSEMINATION/COMMUNICATION OF SCIENCE

Common Techniques for Communication

Science communication has been defined as "the use of appropriate skills, media, activities, and dialogue to produce one or more of the following personal responses to science: Awareness, Enjoyment, Interest, Opinion-forming, and Understanding" (Burns et al., 2003).

Scientists are not only asked to communicate their findings inside and outside academia, but also to build bridges between research and the society at large and, more importantly, to engage the general public, developing a bi-directional and critical dialogue with the different categories of social actors, (i.e., stakeholders).

Dissemination of scientific results to different target groups is increasingly recognized as a responsibility of scientists (Brownell et al., 2013) that needs the support of other professionals, e.g., journalists, artists, Information Technology (IT) specialists and social networks managers (Uyarra and Borja, 2016). Awareness of the need for better science communication has grown enormously over the last 40 years. The communication of science to different target groups, including the society at large, and the transfer of knowledge is now required in research programmes. Science plays a central role in our life, so policy makers and the wide public are not be able to make informed decisions without understanding the scientific basis (Treise and Weigold, 2002; Fischhoff, 2013).

Science is mainly financed through public funds. Worldwide, numerous organizations (e.g., governments, agencies, foundations) and a large diversity of research programmes are in place to fund research and innovation [e.g., Horizon, 2020 European Union (EU) and National Science Foundation (US) programmes]. Both human and economic resources are being used to this end. Therefore, bridging the gap between science and policy through effective dissemination is a must for such funding programmes to be considered as useful and successful. Although some progress that has been made in disseminating health research output to bridge the gap between science and practitioners (Wilson et al., 2010; Neta et al., 2015), this does not apply to most fields of research. Whether research outputs reach the relevant target groups (e.g., society, consumers, specific economic sectors, decision makers, policy makers, etc.) is yet not well-studied, but it is crucial for societies to become more knowledgeable and reach a better capacity to make informed-decisions.

Indeed, until recent times, not much relevance was given to dissemination and a greater focus was placed on ensuring that scientific outputs were reflected in the scientific literature. The potential impact through the development, dissemination and use of project results was often neglected, both in the call for research proposals and the proposals themselves. Many calls for proposals clearly state the need for dissemination activities to increase impact. Science dissemination is now evaluated in research project assessments and constitutes an important criterion to achieve an outstanding and fundable project (Pohl et al., 2010). Furthermore, there is considerable pressure from the funding agencies for scientists to communicate with and to involve society in research through "citizen science." However, despite its importance, guidance on what it is expected from scientists in terms of dissemination is still weak, and little has been developed as to how the success of any dissemination strategy may be measured.

Taking this into account, the aim of this article is to provide guidance to scientists on planning and implementing an effective dissemination strategy. In order to do so, we first provide a brief overview of the EU approaches to the dissemination of science. We then review the most important dissemination approaches, tools and activities available to a science communicator, and report on their effectiveness and on the difficulties that could be encountered. We illustrate this using the experience gained during the EU-funded project DEVOTES (DEVelopment Of innovative Tools for understanding marine biodiversity and assessing good Environmental Status; http://www.devotes-project.eu). In this project, the consortium prepared a dissemination strategy during the planning phase of the project that aimed at maximizing the impacts of the research. We (the Dissemination Team of the DEVOTES project) have collated a number of theoretically and practically informed frameworks that could be used by other scientists as a guide for planning and accomplishing a fruitful dissemination of their project results and outputs, both at the European and the international level.

The Importance of Science Dissemination for the EU

Over the last decades, the European Commission's economic policy has largely been based on the belief that progress and economic growth are achievable through techno-scientific knowledge and innovation (PotoČnik, 2007). Therefore, if society understands the critical role that science and technology plays, public support should follow naturally. The nature of the science-society relationship has shifted since the 80's, but the idea still lies at the heart of Europe's strategy. Back in the late 1980's, science-society issues were considered a problem that could be solved by increasing classic communication efforts. The paradigm "*Public Understanding of Science*" (Royal Society, 1985) regarded the communication model as a linear function, where dissemination efforts would fill the knowledge gap and would make citizens supportive of science and technology policies.

The 1990's and EU Framework Programme 5 (FP5) were oriented to "*Raising Awareness*," which stressed that researchers should increase their involvement in dissemination activities. Moreover, through the Marie Curie Actions and the launch of gender mainstreaming (European Commission, 2001), more effort was made to attract Early Career Scientists and women into research.

At the beginning of the millennium, the key concepts of "dialogue" and "participation" were introduced, anticipating new ways of governance in science and technology. The EU FP6 funded the "*Citizen and Governance in a Knowledge-Based Society*" and "*Science and Society*" calls. The latter was modified to "*Science with Society*" in FP7, with the aim of improving linkages between science and society. This stressed the idea of considering science and society as a single entity, increasing the role of the wider public and non-research actors in science policy making, and making the results of publicly funded research more accessible (Wilkinson et al., 2016).

The last step in the recent evolution of the European science communication strategy is constructed around "*Innovation Union 2020*," where innovation is seen as the key tool for strong and sustainable growth. In this framework, the Responsible Research and Innovation (RRI) concept implies that all societal actors (e.g., researchers, citizens, policy makers, third sector organizations, etc.) work together during the research and innovation process to align its outcomes with the needs, values and expectations of society. One of the key pillars of Horizon 2020 is "*tackling societal challenges that are important to all EU citizens and can have a real impact benefitting the citizens.*" These benefits include:

(i) Health, demographic change, and well-being;
(ii) Food security, sustainable agriculture and forestry, marine, maritime and inland water research, and the Bioeconomy;
(iii) Secure, clean, and efficient energy;
(iv) Smart, green, and integrated transport;
(v) Climate action, environment, resource efficiency, and raw materials;
(vi) Europe in a changing world—inclusive, innovative and reflective societies;
(vii) Secure societies—protecting freedom and security of Europe and its citizens.

In summary, the European view on science-society issues has evolved from considering science as a source of rarely questioned knowledge, to a practice deeply intertwined with society (ESF Science and Policy Briefing 50, 2013).

In 2013, the European Commission's launched Horizon 2020 (H2020), a research and innovation programme that will run from 2014 to 2020. H2020 supports scientific research and innovation with an overall budget of approximately €80 billion (European Commission, 2013). The H2020 Communication guidelines (European Commission, 2014) provide a checklist to guide the participants in building a communication strategy specific for their project. This includes guidelines for:

(i) The good management of resources and people in the dissemination of results, which implies a dedicated work package in the proposal, the preparation of a dissemination plan, the allocation of an adequate budget and the involvement of professionals in the field of science communication;
(ii) A series of activities to ensure the continuity of the dissemination after the end of the project;
(iii) Well-defined goals and objectives for the dissemination, with specific deadlines and evaluation criteria to measure its efficiency and impacts;
(iv) A well-defined audience and specific target groups;
(v) A distinct communication strategy and dedicated dissemination means for each target group.

Communication Tools

There are various approaches to communicate scientific findings, ranging from more formal (e.g., academic activities, lectures, seminars, production of textbooks, SCI publications) to informal activities (e.g., exhibitions, documentaries, media programs, science clubs and societies, educational games, theater performance, open lectures, festivals, magazine articles, and internet-based tools such as websites, blogs, social media, podcasts, newsletters; Burns et al., 2003). Scientific journalism has traditionally been used as the main format for the communication between science and the public, with the aim of filling in the gaps in the knowledge of the society at large (Treise and Weigold, 2002). However, not all topics are equally covered, and around 70% of scientific journalism coverage is on medicine and health. Scientists used to communicate their results in two main ways: (i) publishing in peer-reviewed journals, and (ii) presenting their findings at conferences. Both these methods are mainly directed to other scientists as most of the scientific journals are accessible only through institutional subscriptions, and conferences are mostly attended by other researchers. More recently, scientists have started to use Internet and social media as means to directly communicate. Innovation in new technologies has led to the development of new approaches, which not only encourage the dialogue between scientists and the general public, but also stimulate people to have an active role in science. In this sense, social media has helped science communication to transform itself from a one-way to a two-way system, where users interact directly with the scientist (**Figure 1**). In addition, citizen science (i.e., the active engagement of general public in scientific research projects, often acting as collectors of data) and crowdfunding (i.e., the request by founders of for-profit, cultural, scientific, and social projects to request funding from many individuals, often in return for future products or equity; Mollick, 2014) are now becoming more and more important in research projects development.

The Dissemination Experience of DEVOTES

DEVOTES is a EU FP7 collaborative project involving 22 partners distributed across 14 countries in the Atlantic Ocean,

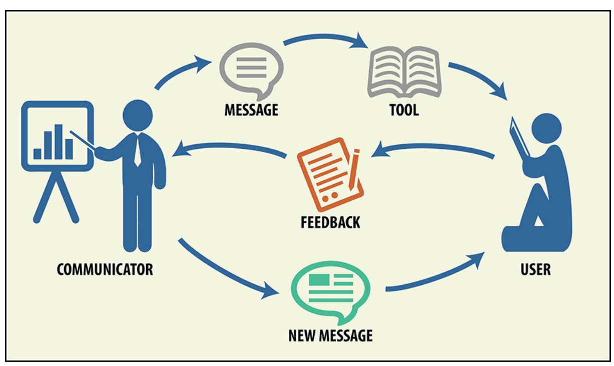

FIGURE 1 | Two-way dissemination approach.

and the Baltic, Mediterranean, Black, and Red Seas. DEVOTES was developed with the main objective of improving our understanding of the relationships between anthropogenic pressures, their influence on the climate and their effects on the marine environment. The project was funded for improving and/or enhancing the effectiveness of ecosystem based management (EBM) in order to fully achieve the Good Environmental Status (GES) of European marine waters, in the context of the European Marine Strategy Framework Directive (MSFD; 2008/56/EC). To achieve this goal, DEVOTES developed a wide set of innovative indicators, models and tools to assist in the characterization, quantification and assessment of marine biological diversity, non-indigenous species, food-webs and seafloor integrity status at an European scale.

The communication strategy of DEVOTES was developed during the preparation of the proposal, with the main aim to build a network with the stakeholders and to provide an effective dissemination of the project achievements. The dissemination activities included an interactive communication dialogue with stakeholders, policy makers and society at large, as well as a uni-directional communication of results. In addition to the traditional approach of dissemination, (e.g., publications, presentations in conferences, organization of workshops, documentaries, etc.), DEVOTES made an effort to define the use and development of new tools to actively involve the different target groups, through the development of apps and the use of social media.

All the planned dissemination activities were directed to achieve the main objectives of DEVOTES. These included building knowledge of the functioning of marine ecosystems (i.e., promoting Ocean literacy, see Uyarra and Borja, 2016), and raising the awareness of the implications of human activities on marine ecosystems. Without this solid understanding, the public cannot make informed decisions and respond in an efficient and timely manner to solve environmental issues.

The next two sections will describe the activities carried out during the lifetime of DEVOTES to disseminate results and progress, and will analyze the performance of each tool.

DISSEMINATION APPROACHES

Communication Strategy and Dissemination Plan

Effective communication enhances the impact of a project and the possible uptake of the results. Therefore, the communication strategy of a research project should be discussed in detail and the various phases of the communication strategy should be established during the development of the project proposal. These phases include capturing public interest about the topic, disseminating the project results and outcomes, and finally ensuring and communicating the legacy of the project. The chosen communication approaches should also be established at this stage, as should be the identification of the target audiences.

The different inter-related phases for an effective communication strategy in a research project were taken into account in DEVOTES: the development of a dissemination strategy and plan, and the identification of key reporting elements and of the cross-cutting issues (**Figure 2**).

The communication strategy should be developed by a small communication team that includes, at least, the project coordinator, the webmaster, the graphic designer, and one scientist in charge of the dissemination. The inclusion of additional professionals, such as a scientific journalists and artists would be beneficial to this team. In addition, and to ensure that all work carried out within the project has the potential for equal visibility, each work

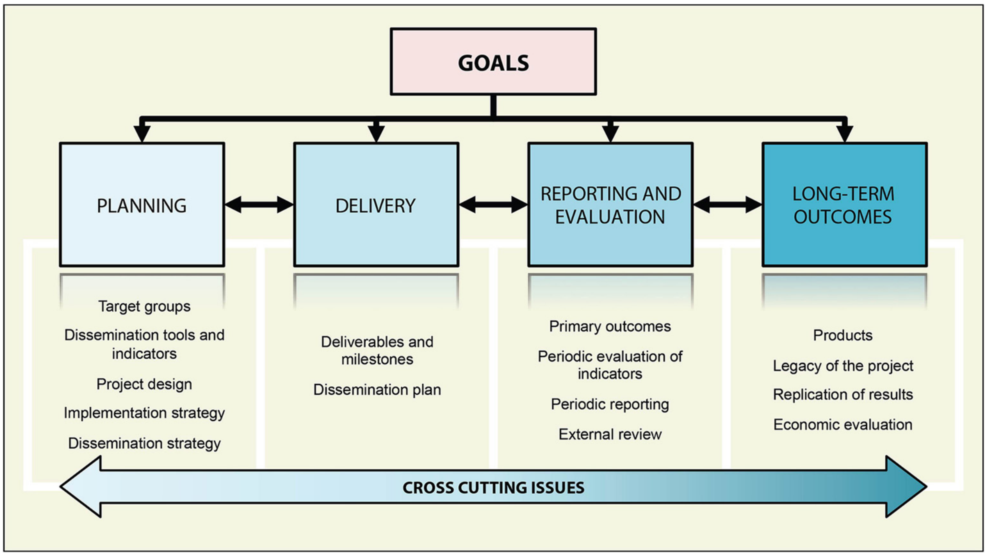

FIGURE 2 | Framework for enhancing the value of DEVOTES research for dissemination and implementation (inspired and adapted from Neta et al., 2015).

package of the project should nominate their communication officer, who will be the contact point for the communication team.

A time-line must be established for the various phases of communication in line with the timing of deliverables, and considering the necessary time-lag to prepare for the dissemination product linked to the specific deliverable (objective) and target audiences, which should also be defined. Once the communication strategy has been discussed, the communication team should draft a dissemination plan. The dissemination plan is a document that is revised at 6 months intervals throughout the duration of the project. It serves as a guide to the communication team and other project members to outline the actions, product outputs and target audiences to be reached during the project. The lead partner(s) for the different actions are also identified. The dissemination plan is a "living document" that can be revised and adapted to accompany the project development. During the project, the details of the various actions that have been undertaken may be added so that the dissemination plan is slowly transformed into the dissemination report as the project is implemented.

The dissemination plan should be structured to include the following sections, although others may also be necessary: (i) an executive summary; (ii) the target audience(s); (iii) the messages; (iv) the tools and mechanisms; (v) the calendar including the post project legacy; (vi) the assessment and monitoring; (vii) the indicators for the evaluation of the dissemination goals, and (viii) the internal communication. Moreover, a SWOT (Strengths, Weaknesses, Opportunities, and Treats) analysis should be included and revised during the project (**Figure 3**). The SWOT analysis is a structured planning method that identifies the internal (strengths and weaknesses) and external (opportunities and threats) factors that are helpful or harmful to achieve a specific objective, and can be a useful tool to evaluate the dissemination strategy of a project. The results of the SWOT analysis determine what may assist the dissemination team in achieving its objectives, and in identifying what obstacles must be overcome or minimized to achieve foreseen results. Additionally,

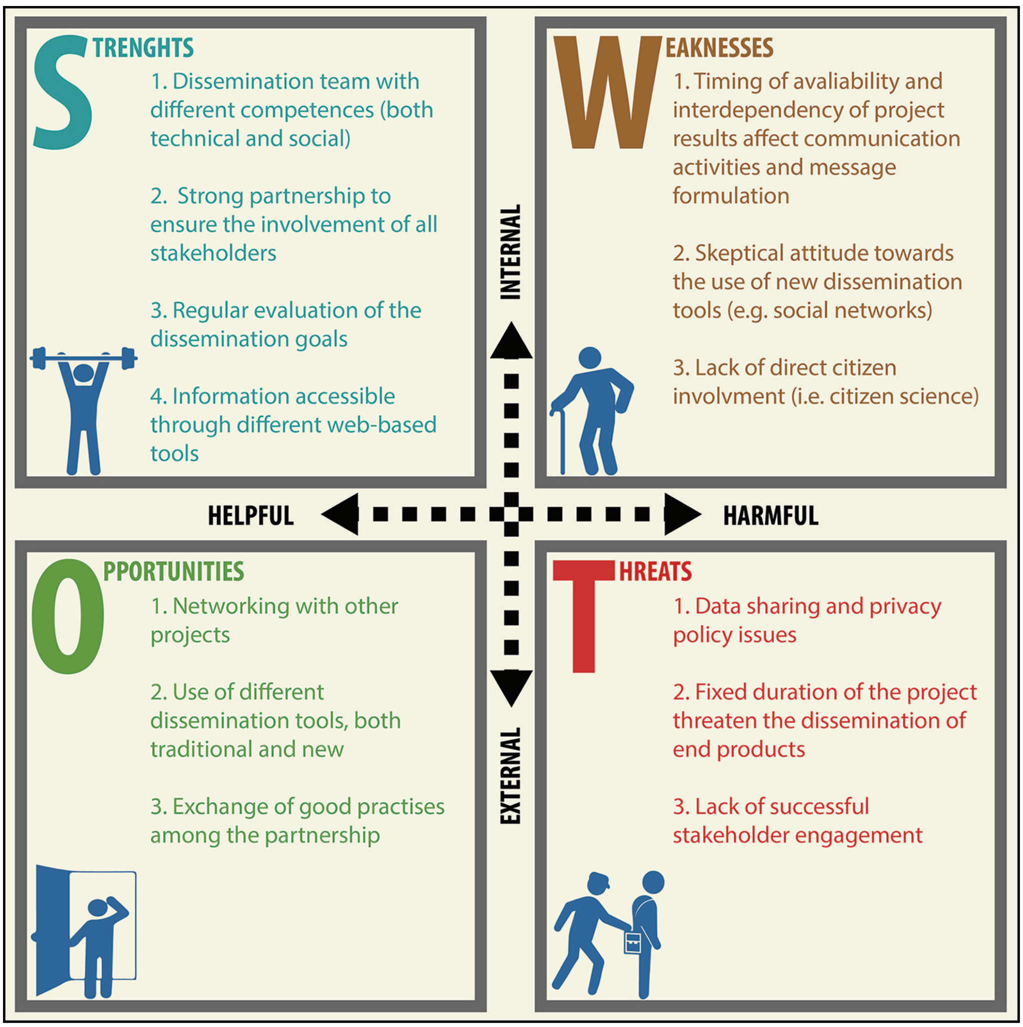

FIGURE 3 | SWOT analysis of the DEVOTES Dissemination strategy.

annexes can be added in the dissemination plan containing tables with details about the venues, participants, link to the products and other pertinent information. Other annexes may include examples of posters, leaflets, and other materials.

Dissemination actions should be targeted at well-defined audiences. The results of a research project may be of interest to the general public, but also to specialists and high-level policy makers. Different means and media of dissemination, vocabulary, and message are appropriate for each of these categories. This audience needs to be informed about the project, its progress, its results, its outputs and its legacy.

In order to maximize the impact of a research project, it is important to engage with all interested parties and communicate the results of the research. "Interested parties" include a wide variety of stakeholders, as well as the "end-users," i.e., those who will be able to make use of the findings, outcomes, and products. For the results to be useful, they should be of interest and easily accessible. Ideally, the identified end-users engage with the project at the design stage. Co-design allows end-users to actively participate and communicate their interests, and help the scientists to co-develop the project so as to maximize its uptake and legacy.

Engaging with the stakeholders can be surprisingly difficult, due to insufficient funds to engage them dynamically resulting in "stakeholder fatigue," because of the multiple requirements both from the project and from other projects on similar topics. There are existing guidelines about stakeholder engagement, such as Durham et al. (2014). For a balanced viewpoint, it is important to engage with different types of stakeholders and to establish a solid discussion with end users and local stakeholders (Saint-Paul and Schneider, 2016).

DEVOTES Dissemination Strategy

The DEVOTES Dissemination Team developed its communication strategy during the negotiation phase of the grant and requested that each partner nominate a responsible for the dissemination. Dissemination influences the decision-making process, and therefore the first step is to identify the audience, listen to it, identify which decisions are required and therefore what information is necessary (Fischhoff, 2013). The DEVOTES Dissemination Team therefore first focused on building a stakeholder map, identifying the audience and the specific targeted messages, the mechanisms of communication and finally defining a specific timeline for the different activities.

Besides the general public, another six categories of stakeholders were identified as target groups of dissemination, through an analysis of the characteristics of the audience engaged with DEVOTES project: (i) scientists with interest in marine monitoring, biodiversity, and assessment, (ii) higher education institutions, (iii) environmental agencies and/or other institutions operating at the national and regional levels, (iv) decision making authorities, (v) environmental associations, NGOs, fishing, and aquaculture associations, maritime transport associations, port authorities, and (vi) private and industrial stakeholders, including Small and Medium Enterprises (SMEs). The dissemination approach included a strong web presence through a dedicated website, social network accounts, and e-newsletters, participation in conferences and fairs, publication of scientific papers, organization of training activities and networking with other EU funded projects.

The DEVOTES Dissemination Team, with the contribution of all partners, created the database of stakeholders, which now includes more than 1500 contacts in marine environment research and industry. All were contacted early on to introduce them to the project concept through unidirectional communication, emails and the distribution of the electronic newsletter.

Traditional Tools

The identification of the audience potentially interested in DEVOTES results and the categorization of the different stakeholder groups were fundamental for the dissemination planning: for each audience cluster identified in the stakeholder map we used dedicated dissemination tools (**Figure 4**). Statistic information about the use of these tools is discussed in Section Evaluation of the Dissemination Goals of this paper.

The Dissemination Team held regular meetings to revise the plan and adapt it to the progress of the project. This resulted, for example, in a deep revision of the homepage layout and website structure 2 years after the beginning of the project and on the participation in Regional Sea meetings rather than organization of workshops.

The website

Nowadays, the Internet is the primary medium of science communication (Kling and McKim, 2000), and web-based communication is crucial for engaging public audiences with science (Bultitude, 2011). The DEVOTES dissemination strategy included various Internet-based tools the foremost of which was a dedicated website, http://www.devotes-project.eu, used as the main communication channel for the project management, achievements, and progress. A special effort of the Dissemination Team was focused on developing an eye-catching layout and a user-friendly website map. The website, dedicated to all stakeholder categories, was developed by graphic designers, under the supervision of the project coordinator and in accordance with the EU guidelines. The website has been constantly and timely updated with news, promotional material and new project products. The site map included six main sections:

(i) About the Project, to introduce the project objectives, the work plan, and the partners involved;
(ii) News and Events, to promote the research progresses, project meetings, and conferences on topics related to DEVOTES and other EU funded projects events;
(iii) Research Outputs, to promote and provide easy access to scientific publications, reports, and tools developed during the lifetime of the project;
(iv) Young Scientist Corner, to present early stage career researchers working in DEVOTES [with the series of interviews ("Ph.D. students of the Month") and to promote training and job opportunities within and outside the project];

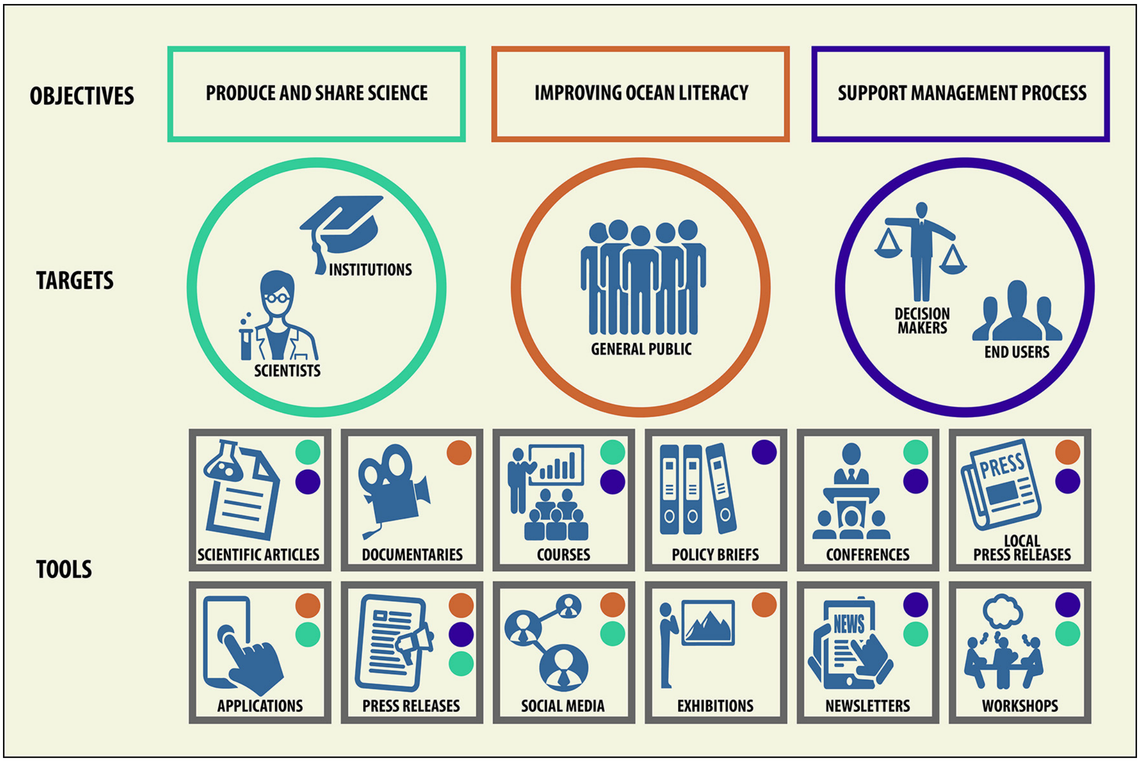

FIGURE 4 | Targeted dissemination tool for each macro audience cluster identified in the DEVOTES stakeholder map. The targets to which each tool is dedicated are reported in order of importance: e.g., scientific articles are mostly directed to scientists (green circle) and secondly to decision makers and end users (blue circle); documentaries are instead mostly directed to the general public (orange circle).

(v) Media Center, to make available the promotional material; and
(vi) Partners' Area, to facilitate the communication within the consortium.

A full set of informative and promotional material, including factsheets, policy briefs, brochures, and posters, was produced during the lifetime of the project to promote the release of reports, software tools and deliverables. All the promotional products, the website and templates (for presentations, posters, reports, minutes of meetings) were developed using the corporate image of the project, always including the DEVOTES logo and using a consistent color code.

Special attention was dedicated to the early career researchers (ECR), within and outside the project: the Young Scientist Corner included a series of interviews "*PhD students of the Month*," as well as announcing job opportunities, post-graduate modules, summer schools, and training activities.

The newsletter and email campaigns

The dissemination campaign of DEVOTES was launched with the publication of press releases in the countries of the members of the consortia. This was followed by an email campaign presenting the project and launching the website to all the potential stakeholders. The mailing campaigns continued with a regular electronic newsletter (approximately every 6 months), brief news (every 3 months), and monthly updates on the project progress. All the issues of the newsletter have been made available for download on the project website and promoted via the project social networks.

To enhance the communication inside the consortium, distribution lists were created at Work Package and Task level, for the General Assembly, the Steering Committee members, and for Advisory Committee members. Moreover, in addition to the Partners' Area of the website, a sharing platform has been included among the e-media tools available for the participant to the project.

Scientific publications

In order to better communicate the scientific results, not only within the scientific community but also to decision and policy makers, all the scientific papers produced in DEVOTES have been made Open Access, either with the gold road, paying the fee for the open access, or with the green road, self-archiving the article. As indicated above, academic institutions subscribe to the different journals, but usually they can only afford the subscription to a small fraction of them. This situation decreases the potential usage and impacts of research, which would be maximized if all research papers were Open Access (Canessa and Zennaro, 2009). Open Access enhances the research cycle, improves the access to international research outputs and the impact of the research. There is a correlation between Open

Access publication and citation-count, increasing this from 50 to 250% (Canessa and Zennaro, 2009). Additionally, articles in Open Access are immediately available for free consultation and download and, more importantly, permanently preserved in journals digital archives.

The Dissemination Team created a repository of scientific papers produced during DEVOTES life, named "FP7 EU DEVOTES Community" in Zenodo, the OpenAIRE "orphan repository" available under the link https://zenodo.org/collection/user-devotes-project. With this repository DEVOTES is accomplishing one of the most important objectives of the FP7 Programme, which is the free access to all the research outcomes to scientists and public at large. In addition to Zenodo, the Dissemination Team created a Google Scholar profile for DEVOTES in which all papers are listed, (https://scholar.google.it/citations?user=oSH2JTkAAAAJ&hl=it&oi=pll). This allows scientists to easily obtain information on all the papers published by the project, consult the citations received by each paper, rank them, and obtain the H_{index} of the project, as an index of the success of the project scientific outcome.

As Open Access publications lead to wider and more efficient dissemination of information, the dissemination strategy of DEVOTES included also the production of an ebook, reporting the scientific results and products developed during the project. The ebook, composed by the articles published in this Research Topic will be freely available for download from the website of the project. Moreover, the ebook will be part of one of the applications for smartphone, which will be available by the end of DEVOTES project (October 2016).

Workshops and participation to conferences

The engagement of stakeholders is crucial to reach the objective of generating improved interfacing mechanisms in the management process, among science, policy, and decision makers and the general public. This can be achieved through targeted workshops, conference sessions, and webinars. Once more, the dissemination has to be tailored to the audience. The scientists working in related fields and projects are more easily reached at special sessions in conferences. Practitioners working at environment agencies, either regional or national are best reached through specially organized workshops, if possible using locally relevant materials as examples. International practitioners, such as the Regional Seas Conventions, European Environment Agency and expert groups (e.g., "Good Environmental Status working group"), are best reached at workshops back-to-back with pre-organized meetings. This both increases the likelihood of participation and reduces travel expenses. It is essential to distribute targeted information that explains the workshop well in advance of the meeting, so that the attendees may register and prolong their stay to participate.

Companies and SMEs are more difficult to contact as a group. Environmental consultancy firms may be in competition with each other, and so reluctant to have a joint meeting, and it may be therefore necessary to have individual or small group meetings. However, it was easier to organize group workshops and meetings for other potential end users, for example aquaculture firms that rely on marine good environmental status.

Documentaries

Films and documentaries are one of the most powerful communication and educational tools (Barnett et al., 2006; Hooper et al., 2011), engaging the public in critical thinking and enhancing public awareness in environmental issues (e.g., climate change, pollution, acidification). The production of documentary films has grown significantly in the past decade, and the distribution of documentaries through the Internet created new opportunities to create societal impact (Karlin and Johnson, 2011). Platforms such as YouTube, iTunes, and Vimeo make online videos easier to be made available, accessed, used, and shared. With the aim of increasing the potential impact of DEVOTES, the dissemination strategy included the preparation of a documentary illustrating the background and the main results of the project. DEVOTES was selected by "*Futuris*," the award-winning program of EuroNews on European science, research and innovation, as a successful example of project studying the effects of human activities on marine ecosystems, to raise general interest about the environmental status of European seas. The episode "*Improving our understanding of our seas*" went on air for 1 week and was then made available on the programme EuroNews YouTube channel. The DEVOTES documentary prepared by the project team will be ready in October 2016. A professional company (partner of the project) worked on the details of the storyboard, collecting videos, interviews and images from the DEVOTES partners. It will be broadcast via Internet-based channels (YouTube, Vimeo), available from the project website and promoted via the project social network accounts. A wide audience will be reached by the use of e-media tools for the promotion of the film to increase the social impact.

Training activities

Training activities and summer schools are an important part of dissemination. They provide for the legacy of a project by disseminating the project results to end users, such as postgraduate students and practitioners. Whereas students enrolled in postgraduate courses may benefit from taught modules, practitioners usually do not have the time or professional freedom to enroll in long-term training courses. Focused and short summer schools therefore provide an important opportunity for practitioners to learn complementary skills. The uptake of scientific results published in scientific papers and text books into curricula usually has a long time lag, sometimes lasting several years. Hence, including the training into postgraduate and summer schools, which can be attended by practitioners, fast-tracks the information to current end-users and those about to enter the job market (postgraduates).

A successful training course should be disseminated to potential end-users in a timely manner. In this way, interested candidates can plan to attend, if they are fully employed, or plan to select the course if they are post-graduates. The information provided should include the necessary context so that the candidate understands what training will be on offer and why they would benefit from attending. The training programme should include the knowledge and skills that will be learned when completed.

In the DEVOTES project, the consortium organized four summer schools to disseminate current "hot topics" addressed throughout the life of the project by the different partners. The topics covered were: genomic tools applied to monitoring; new modeling applied to assess the status of marine systems; innovative, and integrative ecosystem quality assessment tools; and ecosystem services provided by seas. DEVOTES Summer Schools have attracted both early career and senior researchers alike. Keynote talks were given around the specific topics listed above. Unlike the classic symposium format, where attendants are exposed to many but very short presentations, the longer length of the talks in these Summer Schools allowed the speaker to extensively expose different aspects of the subject and disseminate the results of the project in detail.

In addition to the primary dissemination and training tasks, these summer schools had other important objectives: (i) networking with scientists not involved in the project, either as professors or attendees, to bring fresh ideas into the project tasks and deliverables; (ii) give the opportunity to managers, Ph.D. students, Post-Doc, and scientists attending the school to learn about emerging concepts that can be incorporated into their daily research; (iii) disseminate the findings among more ample communities, e.g., through the collaboration with organizations such as EuroMarine, an European marine research network (http://www.euromarinenetwork.eu); and (iv) publish position papers on the topics addressed, which can be a direct (e.g., Borja et al., 2016) or indirect (e.g., Bourlat et al., 2013; Piroddi et al., 2015) result of the school. The Summer Schools have spread the findings of the project to an ample audience, covering more than 30 countries from all continents. A qualitative analysis of the Summer Schools is reported in Section Impact Analysis.

In addition to summer schools, other ways of training have been explored and implemented in DEVOTES. The use of webinars (online live courses) has been used as means to train on specific topics. As indicated above, there is often interest for learning but difficulties in accessing such knowledge. In the case of physical courses, this might be difficult for those working full time or having limited time or economic resources. To overcome such issues, webinars can be a realistic solution. In DEVOTES, webinars have been used to train key stakeholders on the most relevant tool developed under the project. With a total participation of 76 relevant stakeholders, and feedback received, it can be considered a very cost-effective means for communicating and practical training. The webinars are also available on the website of the project, together with short, YouTube training videos, and guidelines.

New Tools

Social media

Internet platforms, mobile applications (Apps), and social media have now also become resources to share research progress and to learn. All these tools represent a unique opportunity for scientists to enhance ocean literacy, "*understanding of the ocean's influence on you—and your influence on the ocean*," (Carley et al., 2013), allowing citizens to take informed decisions and to be able to participate in public debate about ocean health (Fauville et al., 2014).

Generic and professional social media tools, such as ResearchGate, LinkedIn, Facebook, Twitter, or Instagram have exploded in popularity in the last decade, attracting more and more scientists to using them. As mentioned above, online presence is fundamental for science communication and, together with social media, offers a wide range of benefits for scientists: boost their professional profile, enhance professional network, improve research efficiency and scientific metrics (Bik and Goldstein, 2013; Jucan and Jucan, 2014). Using social networks to promote research results and paper publications has been proved to increase the number of citations of their articles and the H_{index} (Liang et al., 2014). A strong presence on social media may result in papers having 11 times more possibility to be cited vs. articles lacking of social media presence (Eysenbach, 2011). Additionally, generic social networks offer the opportunity to reach a wide range of people with a more or less developed personal interest in science and to develop that interest (Fauville et al., 2014).

DEVOTES has been present on a few, carefully selected social media tools, both professional and generic, to take advantage of the specific features of each one (pros and cons of the different media tools will be discussed further in Section Comparison of Different Media Tools). The DEVOTES Dissemination Team created an account and a discussion group in LinkedIn, with 206 members, which served as tool to improve sharing knowledge with other scientists and industry professionals in the marine and environment fields, to enhance the ocean literacy among these two target groups. DEVOTES made its social debut early in 2013 (ca. 6 months after the beginning of the project), using the most popular platforms: Facebook, https://www.facebook.com/Devotesproject/), Twitter (@DEVOTESproject), and YouTube. The social media campaign included publishing posts at least three times per week from the project and project coordinator accounts.

To make DEVOTES appealing for the general public and decision makers, the DEVOTES Dissemination Team published posts on the website and social networks on environmental days (e.g., the 22nd March World Water Day, 8th June World Oceans Day), linking the project activities with the topic of each day. For example, on the International Day of Biodiversity (22nd May) we linked its topic "*Mainstreaming Biodiversity; Sustaining People and their Livelihoods*" with the main message of the DEVOTES Final Conference: "*Marine biodiversity is the key to healthy and productive seas*."

Other messages were dedicated to different categories of stakeholders (e.g., environmental agencies, consulting companies) and therefore included more technical aspects, such as the production of the Catalogue of Monitoring Networks and the development of NEAT, the Nested Environmental status Assessment Tool.

Mobile apps

The innovation in mobile computing technologies and their affordability make the learning process possible using mobile applications ("apps" hereafter). Small devices, such as smartphones and tablets, are now part of our daily life, have strong computing power and they are potentially always connected. Applications for smartphones and tablets are considered useful communication tools, which are able to reach out further than our scientific reports and publications do, including society at large (Hsu and Ching, 2013). Therefore, mobile devices represent a great opportunity for education, science communication and ocean literacy. To this end, the DEVOTES dissemination strategy included the development of mobile applications. Two apps already available are "DevoMAP" and "MY-GES." Another two are planned to be released by October 2016. All apps will be available for iOS and Android devices and downloadable from the project website. DevoMAP and MY-GES aim to disseminate the results from innovative modeling to a wide audience, and to attract the attention of the public, including scientists involved in assessments of GES in European regional seas and those not involved in marine environmental assessments. "DevoMAP" focuses on people directly involved in research and policy, to support the implementation of the MSFD. "MY-GES" targets people interested in our achievements among the general public. By targeting the general public, we aim to make society aware about the Marine Strategy Framework Directive, its implementation and assessments of environmental status. The other two apps will focus on the dissemination of overall project findings: "DevoBook," as a result of this issue of Frontiers, and "DEVOTES," an interactive app for the general public, including key questions and findings from all DEVOTES Work Packages and promotional material produced during the project lifetime.

Artistic Elements

The use of arts in science communication is still poor but a study, conducted by Curtis et al. (2012), showed that ecologists are willing to use the arts in a scientific forum to promote their results. In particular, they think that the visual (e.g., painting) and performing (e.g., ballets, theater plays) arts can be very useful in communicating scientific information.

In 2015, DEVOTES decided to include a visual artistic element in its dissemination strategy. In collaboration with the EU project CoCoNet (Toward COast to COast NETworks of Marine Protected Areas coupled with sea-based wind energy potential), a calendar was produced to be distributed to the project stakeholders at the end of the year. The topic of the calendar was the MSFD implementation, including an artistic interpretation of the 11 MSFD descriptors of GES, which define how to assess the quality of EU marine systems. Each descriptor was represented in an evocative illustration, associated to each month, and briefly outlined in the explanatory text. December's plate describes an ideal observation system, to monitor environmental quality standards, and integrate the information to assess the status and achieve GES (**Figure 5**).

The Calendar, distributed to more than 800 relevant stakeholders, was also made available for download from the website, and in only 3 months the page received more than 600 visits.

The Importance of Networking with Other EU Projects

Taking into account the integrative view of DEVOTES, it was necessary to collaborate with other international, European and regional projects, creating a strong network across Europe and overseas. The tasks and approaches have been multiple. These include:

- To explore complementarities, in implementing the MSFD, with the STAGES project (http://www.stagesproject.eu);
- To develop conceptual approaches, such as those of the DPSIR (Drivers-Pressures-State of Change-Impacts-Responses), with the VECTORS project (http://www.marine-vectors.eu);
- To promote joint workshops and sessions on aquatic systems assessments, with the MARS and WATERS projects (http://mars-project.eu; http://waters.gu.se);
- To share dissemination channels, such as an artistic calendar of the MSFD descriptors, with the COCONET project (http://www.coconet-fp7.eu);
- To coordinate activities at regional sea level, such as those in the Mediterranean, with the PERSEUS project (http://www.perseus-net.eu/site/content.php);
- To collaborate in knowledge transfer for Blue Growth, with the COLUMBUS project (http://www.columbusproject.eu);
- To promote citizen science, through the MyOSD in the framework of Ocean Sampling Day, with the MicroB3 project (http://www.microb3.eu);
- To share datasets and tools, with EMODNET and MARMONI (http://www.emodnet.eu; http://marmoni.balticseaportal.net/wp);
- To develop and use new monitoring tools, such as the Autonomous Reef Monitoring Structures (ARMS), with NOAA (http://www.pifsc.noaa.gov/cred/survey_methods/arms/overview.php);
- To provide advice in developing regional action plans and best practices for integrated monitoring programmes, with ActionMed.

These interactions have resulted in undertaking a real inter- and trans-disciplinary research (Lang et al., 2012), allowing DEVOTES to go farther beyond the state of the art. This could not have been possible with the resources of only one project.

EVALUATION OF THE DISSEMINATION GOALS

Impact Analysis

The key issue of success of a dissemination tool depends on the ability to supply information and to transfer knowledge to the stakeholders and the potential users (Vermeulen et al., 2009), and then for stakeholders and potential users to use this knowledge. In order to evaluate the success of DEVOTES in terms of public engagement, we present here the quantitative analysis of each dissemination tool discussed above. To assess the performance of the dissemination activities on the web, several analytical tools are being used. All statistical data were regularly analyzed and compared with the impact target identified during

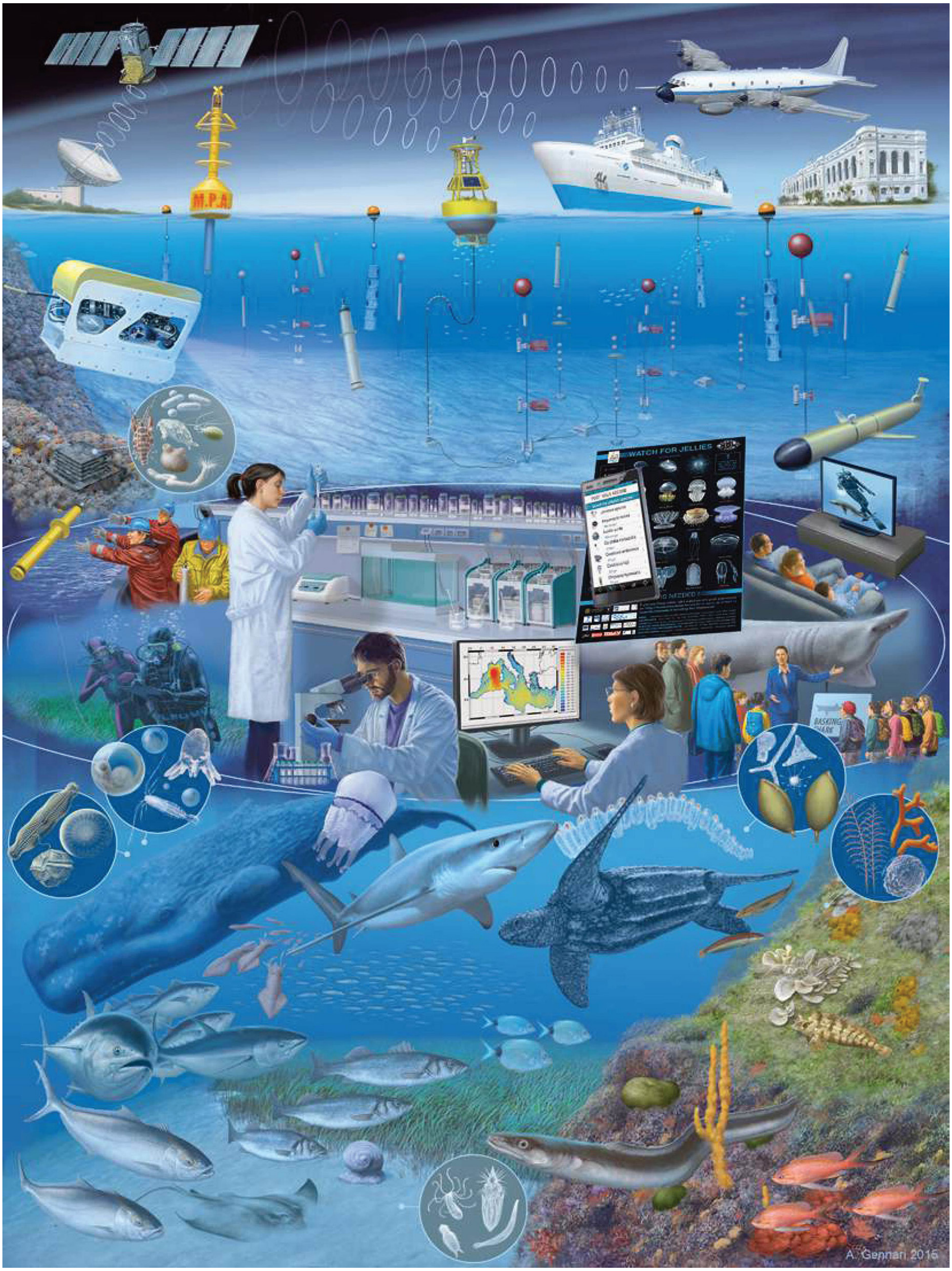

FIGURE 5 | December's plate of the DEVOTES/CoCoNet calendar (Copyright: Alberto Gennari).

TABLE 1 | Impact targets of the main DEVOTES dissemination tools/mechanisms.

Tool/mechanism	Targets/indicators of success	Achievements
Website	3000 individual visitors having visited the website by the end of the project	17700 visitors in 2013; 31000 visitors in 2014, and 49000 visitors in 2015 with an average of 2600 different visitors per month
Newsletter	Six e-newsletters distributed by the end of the project	At the moment of writing, five newsletter issues have been produced and one is planned to be released before the end of the project
Scientific papers	50–75 peer reviewed articles published by the end of the project	139 papers are published after 45 Months
Conferences, International Symposia	80 contributions and four special sessions organized by the end of the project One final conference bringing together stakeholders and scientists	After 36 months, 325 contributions were presented to international conferences and nine special sessions were organized. At the time of writing, the organization of the final conference (Marine Biodiversity—The Key for Health and Productive Seas) is under going
Media activities	At least 9 press briefings and press releases by the end of the project One documentary by the end of the project	More than 10 local press briefing and press releases. At the time of writing, several short videos on DEVOTES activities have been produced and the work of the documentary is running

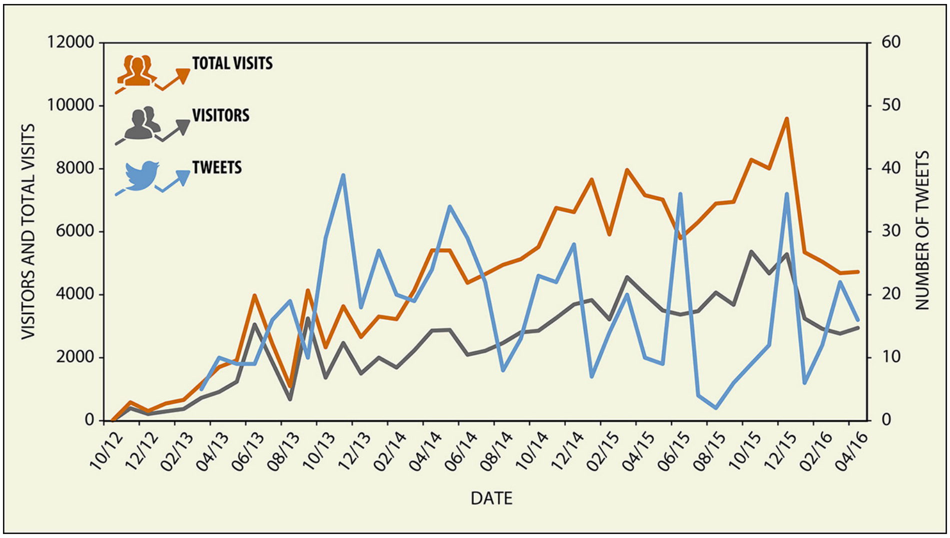

FIGURE 6 | DEVOTES website monthly accesses and social network activity, here summarized as the number of tweets, from October 2012 to April 2016.

the preparation phase of the project (**Table 1**) in order to measure the success and usefulness of the different tools.

To record the accessibility of DEVOTES website, Advanced Web Statistic 7.0 (AWStats, 2010) is being used to analyze the DEVOTES server log files from October 2012 until 2 years after the end of the project. Here, we present the results from October 2012 to April 2016 (**Figure 6**). It can be seen that, besides predictable decreases during summer and holiday seasons, use of the website increased until January 2016. Between January and April 2016, a reduction of the DEVOTES social media presence due to other commitments, led to a decreased interest in the website. An average of 2600 visits have been registered per month, with peaks of up to 10,000 hits during the release of the newsletters (e.g., June, September, and November 2013), the annual meetings (e.g., December 2014 and 2015), the revision of the website (March 2015) and peaks in social network activity (e.g., October 2013). A large proportion of the visitors came from Europe, but the website received visitors also from USA, Africa and Asia. Most of them reached the website via direct link, search engine (i.e., Google) and from external pages (i.e., DEVOTES newsletter and LinkedIn).

In order to evaluate the scientific impact of the whole project, two analytical tools were used to monitor the citations: Google Scholar Citations on the Google Scholar DEVOTES profile, and Altmetric, on the Zenodo DEVOTES community. Google Scholar Citations provide the user with several citation metrics. The DEVOTES papers (139, as of 18th August 2016) have a cumulative H_{index} of 18 and 1083 citations overall. The Altmetric Analytical tool shows the online attention and activity that have been found for each specific article, collecting relevant mentions from social media, newspapers, policy documents, blogs, Wikipedia, and other sources.

TABLE 2 | E-media users in DEVOTES and other EU projects in the framework of Ocean of Tomorrow initiative (FP7-OCEAN).

Project	Twitter	Facebook	LinkedIn account	LinkedIn group	Newsletter
DEVOTES	379 followers	191 likes	184 connections	210 members	Average: 30% of reads
AQUATRACE	115 followers	168 likes	N/U	N/U	N/A
AQUO	————————————No social media presence————————————				N/A
BENTHIS	N/U	422 likes	N/U	65 members	N/A
BIOCLEAN	————————————No social media presence————————————				N/A
CLEANSEA	N/U	321 likes	N/U	51 members	N/A
ECSAFESEAFOOD	128 followers	N/U	N/U	N/U	N/A
KILL-SPILL	————————————No social media presence————————————				N/A
SONIC	————————————No social media presence————————————				N/A
STAGES	————————————No social media presence————————————				N/A

N/A, not available; N/U, not used. AQUO, KILL-SPILL, SONIC, BIOCLEAN, and STAGES do not have any e-media tool (no social media presence).

The E-media analytical tools and results to evaluate the social media impact of DEVOTES are reported in **Table 2**, together with the statistics from other "*Ocean of Tomorrow*" projects started the same year (2012). If we compare the number of social media users, it appears clear that, besides the Facebook page, DEVOTES was able to successfully build its own social community, both in generic (i.e., Twitter) and in professional social media (i.e., LinkedIn).

As the project progressed, there was a positive tendency as more followers (Twitter)/fan(Facebook)/professional-links(LinkedIn) were registered. The traffic on social pages also followed from other dissemination activities, such as the DEVOTES presence in conferences, the organization of summer schools and special sessions, and the participation to global campaigns (i.e., Ocean Sampling Day) and citizen science projects (i.e., My Ocean Sampling Day).

The impact of a successful project dissemination may result in the reassessment and enhancement of the effectiveness of relevant policies, the use of the project results by stakeholders and decision makers, and the creation of business opportunity, as well as s sharing new science-based knowledge.

In order to evaluate the impact of DEVOTES results for policy and decision makers, we monitored the amount of downloads of reports and/or deliverables (**Table 3**). The number of people visiting and downloading some of the reports and deliverables was very high, going far beyond the amount of persons directly involved in the project (around 200).

In addition to these quantitative evaluations, the DEVOTES Dissemination Team carried out also a qualitative evaluation on the Summer Schools and the internal dissemination activities. Satisfaction surveys conducted after each Summer School indicate that attendants were satisfied with the event. From the 61 participants in the Summer School of 2015 who answered to the satisfaction questionnaire, 67% made at least one contact for future projects and general satisfaction was scored with 8.25/10 ($\pm$1.32). However, some of the comments show that attendants were expecting a more interactive format and more opportunities for networking. Therefore, Summer Schools willing to attract students should make an effort to schedule activities with different level of participation.

TABLE 3 | First five products most downloaded from the DEVOTES website (2012–2016).

Product	Date of release	Downloads
Deliverable 1.1 Conceptual models for the effects of marine pressures on biodiversity	June 2014	2497
Deliverable 1.4 Report on SWOT analysis of monitoring	February 2014	1798
Deliverable 3.1 Existing biodiversity, non-indigenous species, food-web, and seafloor integrity GEnS indicators	February 2014	1682
Deliverable 5.1. Report on the set up of the field and experimental activities	November 2013	1670
Deliverable 6.1 Report on identification of keystone species and processes across regional seas	July 2014	1390

Comparison of Different Media Tools

The advancements in information and communication technology are leading to a rapid change in the world of science communication, which is now faster and more interactive. The abundance and diversity of online media sources led to an increased amount of content on offer (Porten-Cheé and Eilders, 2015). Scientists should be present in different arenas and make an effort to interact with the general public. DEVOTES took advantages of different new and traditional media tools (**Table 4**), with the aim of building a "DEVOTES community" which goes beyond the scientific community. If we compare the different dissemination methods used and their performances, it is clear that traditional (e.g., the website) and innovative (e.g., Twitter) tools are strongly related, and that an efficient use of the latter have a positive feedback on the performance of the former. In fact, after our experience in using the different tools during the DEVOTES project, we can rank the different media taking into account their usefulness and cost-benefit: (i) very useful: website, open access publication, sessions at international conferences, stakeholders workshops, Twitter; (ii) useful: summer schools, LinkedIn groups, press releases; (iii) moderately useful: videos, newsletters; and (iv) not very useful: Facebook, smartphone apps.

All the innovative tools should be used as complementary outlet to the traditional tools for the dissemination of new

TABLE 4 | Comparison of different categories of media tools, including pros and cons, together with an evaluation of their usefulness and impact.

Tool	Pros	Cons	Usefulness	Impact
Website	Can allow one-way and two-way of communication	Offline after the end of the project (although in this case, we are working toward ensuring the legacy of the project)	Very useful	Measured only quantitatively (traffic)
	Large potential audiences, composed by all target groups	Need regular maintenance Uncertainty about the type of audience (general public probably difficult to reach)		
Open access of scientific publications (Zenodo, Google Scholar)	Always accessible, broad the citation impact, easy to quantify	Can be only attractive for scientists or very informed people	Very useful	Easy to assess with quantitative citation indices
		Expensive if gold open access		
Press releases	Easy to produce, attract attention to specific issues	Difficult to reach some media	Useful	Easy evaluation of impacts in terms of people reached and the economic value supposed for each media (newspapers, radio, TV, etc.)
		Sometimes mismatch in time between needs of dissemination and interest from media		
Summer Schools	Facilitate networking	Expensive to organize	Useful	Measurable through satisfaction surveys
	Quick way to access young scientists and end-users			
	Ensure training and transference of knowledge			
Sessions at international conferences	Facilitate networking	Require traveling	Very useful	Difficult to assess quantitatively
	Ensure external contrast and facilitate discussions on the project topics with the scientific community	Expensive (registration fees can be too high for early career scientists)		
Stakeholders workshops (including webinars)	Ensure well-focused audience	Physical workshops are expensive	Very useful	Measurable indirectly (assess if the message has reached their meeting's agenda)
	Allow to "individualize" the message	Webinars can experience technological problems (connections, incompatibilities, etc.)		
		Adequate end-users do not attend		
Videos	Facilitate divulgation	Are expensive to produce	Moderately useful	Measured only quantitatively (downloads)
	Can be used in different media (website, YouTube)	Loss of interest very quickly		
Newsletter	Completeness of information	People receive too many emails	Moderately useful	Measured only quantitatively (downloads)
	Targetable	Spam filters		

(Continued)

TABLE 4 | Continued

Tool	Pros	Cons	Usefulness	Impact
Facebook	Established juggernaut in the social media world	Potentially superficial interaction Privacy concerns	Not very useful	Measured only quantitatively (number of friends, likes)
Twitter	Low time investment Ability to rapidly join in on online conversations	Posts are quickly buried under new content Gaining followers can be a slow and difficult process	Very useful	Measured only quantitatively (number of followers). Qualitative analyses can be very expensive
LinkedIn groups	Trustworthy professional platform Allow two-way of communication	Can attract people who want to join as many groups as possible, but do not interact with the group	Useful	Difficult to assess, only by proxies (request to join the group, contact through other communication tools, etc)
Smartphone apps	Users tends to use more apps than spend time surfing a website Available offline Targetable	Complex development process Require maintenance	Not very useful	Measured only quantitatively but downloads would indicate interest in the subject

(Adapted from Bultitude, 2011 and Bik and Goldstein, 2013).

posts from the project website, to share articles, advertise job opportunities, and training events, promote meetings and circulate information about the project progress and results. This should include media that have been shown not to be very useful in the DEVOTES project such as Facebook and mobile apps, reaching audiences familiar with these media. In some cases, the lack of usefulness may be related with the longer time of maturation needed to reach a large audience, such as in the apps. However, not all media tools are necessary: the revision of the dissemination plan and the performance analyses should help to shape the social media strategy, also identifying which tools are redundant (e.g., Facebook and Google+), to avoid overlap. In the case of DEVOTES social media, we decided to focus our attention and efforts on Twitter campaigns, LinkedIn group discussions and website updates, although the Facebook account and the YouTube channel were still active.

Difficulties in Engaging the Stakeholders

Common difficulties encountered during dissemination to the different target group include sharing information between projects, engagement of local stakeholder, copyright, and open access. Researchers have often participated in previous, related projects but may face some constraints about sharing information. For example, contact details of stakeholders may be protected by privacy laws and therefore the effort of stakeholder mapping may have to be repeated. Conference organizers may also face constraints about distributing the contacts of participants. Another constraint is about data sharing. This may result from a number of issues. Often the data may have been previously collected by a team, of which only one member participates in the new project. This person may therefore not be able to share the data as they are not the sole owner of the data. Another typical example is about data format. Data may exist in a different format, and in the case of historical data, it may only be available in paper reports. The transcribing of such data into digital format can be a very onerous and thankless task. Other examples are obsolete storage such as floppy disks, or storage using obsolete software programmes. Trivial examples include different formats such as using a decimal point vs. a decimal comma or apostrophe. Units may also need to be converted, such as concentration in mass/volume instead of molar concentration.

Copyright and open access of information is another common problem. National or internationally funded research often requires that results be publically available or in "open access" format. While many publishers now offer that option, it comes at a price. The project participants may not have budgeted for such costs. A successful project that may publish about 200 articles may have open access costs of more than 500,000 Euros, a significant proportion of the budget. Making articles freely available without using open access, even for research and educational purposes, may infringe copyright laws.

The engagement of local stakeholders, and crucially of possible end-users, can also be problematic. First it is important to identify these potential stakeholders, and then be able to contact them. Once more, even if one project partner has this information, they may not be able to share it with the other project partners. Once the contact details are known, then the stakeholders are best approached personally, rather than through "mass" email messages. The dissemination team should communicate why the contact is considered to be an important stakeholder. How the stakeholder may participate in the co-design of the project at the onset and the project, how they may participate in the product development phase, and finally how the project information may be of use to the stakeholder, are also relevant points.

Difficulties in Engaging the Wide Public

The health and state of our marine environment and the ecological changes being detected and predicted for the future are a global area of interest. No matter how far we live from the sea, the ocean has a strong influence on Human life, providing food energy, moderating climate, and playing an important role in the economic prosperity of many regions. Yet, the common knowledge and understanding of the oceans is not spread enough among the general public and decision makers.

A large part of the general public still obtains their science news from traditional media, such as television, and print newspapers, but internet-based tools are becoming more widely used among teenagers and young adults. Going online regularly and using Google searches now represent the standard approaches for discovering information about a topic (Bik and Goldstein, 2013). However, people feel overwhelmed by the amount of information available.

Another common problem in disseminating EU research project findings is the translation and cultural adaption of the dissemination tools/mechanisms. Most of the material is produced in English, and only selected products are translated into local languages. Moreover, although people think scientists and policy makers should be engaging in dialogue with the public about science, this is not always translated into a willingness to be personally involved. The general public tend to think that is the role of "experts" and not theirs to advise the governments on science issues. However, people show more interest in research and science when they can be directly involved in the project: citizens are more motivated if they can "actively" contribute to science advancements. If people do not see how they can make the difference or being actively involved, they may lose interest. To this end, we suggest that citizen science activities should be included in research project proposals.

CONCLUSIONS

An effective science communication allows people to make sound choices (Fischhoff, 2013) about environmental issues, and help key actors to improve processes and methodologies in marine environment management. From our perspective, the most useful media tools used to disseminate DEVOTES have been the website, the open access publications, sessions at international conferences, stakeholders workshops, and Twitter. Other media could be considered for specific targeted audience.

There are several factors influencing the dissemination of European funded projects, such as the limited project duration (e.g., 2–4 years), which could threaten the dissemination of end products, (see "Threats" reported in **Figure 3**). This in turn could influence the assessment of the dissemination impact to the stakeholders and the general public. To solve these risks, we suggest to include periodic (at least every year) web-based and physical surveys to monitor the effectiveness of results. Additionally, recent studies reveal that, although having a positive view of science and technology, EU citizens think scientific research is difficult to understand and that scientists should be more effective in communicating scientific results (European Commission, 2007, 2010). Our suggestion is to include (where possible) a citizen science initiative in the communication strategy, in order to actively involve the general public, not only in the collection of data but also in the dissemination process (e.g., increasing the social media audience and presence). In fact, the lack of a citizen science initiative was the factor determining the low success of the DEVOTES Facebook page (see "Weakness" reported in **Figure 3**).

Therefore, it is fundamental to develop an effective dissemination strategy at the moment of writing a research project proposal, and to perform a constant evaluation of the dissemination results before, during and after the project lifetime, involving all the key actors, advisory board and partners (see "Strengths" reported in **Figure 3**). To achieve this, the use of different media tools, targeting them to the adequate audience, will ensure the success of the project, by making available all the outcomes and products to the end users.

AUTHOR CONTRIBUTIONS

MM wrote a first draft of the manuscript, then AN, MU, CA, and AB contributed equally to the manuscript.

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Bridging the Gap between Policy and Science in Assessing the Health Status of Marine Ecosystems

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Human activities, both established and emerging, increasingly affect the provision of marine ecosystem services that deliver societal and economic benefits. Monitoring the status of marine ecosystems and determining how human activities change their capacity to sustain benefits for society requires an evidence-based Integrated Ecosystem Assessment approach that incorporates knowledge of ecosystem functioning and services). Although, there are diverse methods to assess the status of individual ecosystem components, none assesses the health of marine ecosystems holistically, integrating information from multiple ecosystem components. Similarly, while acknowledging the availability of several methods to measure single pressures and assess their impacts, evaluation of cumulative effects of multiple pressures remains scarce. Therefore, an integrative assessment requires us to first understand the response of marine ecosystems to human activities and their pressures and then develop innovative, cost-effective monitoring tools that enable collection of data to assess the health status of large marine areas. Conceptually, combining this knowledge of effective monitoring methods with cost-benefit analyses will help identify appropriate management measures to improve environmental status economically and efficiently. The European project DEVOTES (DEVelopment Of innovative Tools for understanding marine biodiversity and assessing good Environmental Status) specifically addressed these topics in order to support policy makers and managers in implementing the European Marine Strategy Framework Directive. Here, we synthesize our main innovative findings, placing these within the context of recent wider research, and identifying gaps and the major future challenges.

Keywords: environmental status, marine health, status assessment, management, ecosystem approach, socio-ecology

INTRODUCTION

A recent assessment of marine ecosystem ecology identified eight grand research challenges (Borja, 2014): (i) understanding the role of biodiversity in maintaining ecosystem functionality; (ii) understanding the relationships between human pressures and ecosystems; (iii) understanding the impacts of global change on marine ecosystems; (iv) developing integrative assessment of marine ecosystem health; (v) ensuring delivery of ecosystem services by conserving and protecting the seas; (vi) understanding the way in which ecosystem structure and functioning may recover through restoration; (vii) understanding the need for an ecosystem approach and integrated spatial planning in managing ocean use, and (viii) developing better ecosystem models to support more effective management.

These challenges reflect widespread recognition of clear effects of pressures from established and emerging human activities on marine ecosystems (Halpern et al., 2015) and, consequently, the potential of those pressures to alter the ability of ocean ecosystems to provide services that yield societal and economic benefits (Barbier et al., 2012; Turner and Schaafsma, 2015). Given the multiple pressures society places on marine ecosystems and the broad range of services they provide, a holistic assessment (Borja et al., 2016) of the status of marine ecosystems requires scientific evidence-based Integrated Ecosystem Assessments (IEA; Levin et al., 2009). Indeed, the former European Commissioner for Environment, Janez Potočnik, stated during the closing session of Euromares 2010, on the occasion of the European Maritime Day, that: "*We are learning that the* [Marine Strategy Framework] *Directive has a weakness—and that weakness is the lack of knowledge*." With a lack of knowledge "*...these unknown variables pose a real problem for decision-makers. They need to be identified and addressed in a systematic way. And while we need to acknowledge the differences and diversity of our seas, there are some issues which can only be adequately addressed on a European scale.*" These statements capture the desire of policy-makers and managers worldwide to fulfill their moral mandate to conserve and protect the seas (Reker et al., 2015) using evidence-based decision-making. Hence, the vision for clean, healthy, biodiverse, and productive oceans and seas with sustainable resource use requires bridging the gap between policy and science in assessing the status of marine ecosystems by increasing scientific knowledge of marine ecosystems and their functioning, including humans and their role as part of the ecosystem (Borja et al., 2013). Indeed, recent European and national policies enshrine the vision of healthy and biologically diverse seas (e.g., DEFRA, 2002; European Marine Board, 2013). More recently, the European Union and United Nations have tried to address problems associated with exploitation of deep fishing resources and associated impacts on biodiversity (St. John et al., 2016).

The development and implementation of policy and legislation globally demonstrate a significant effort to improve the status of the seas, including an ecosystem approach to ocean use management (Browman et al., 2004; Nicholson and Jennings, 2004; Borja et al., 2008, 2016; Curtin and Prellezo, 2010). In the European Union (EU), the Marine Strategy Framework Directive (MSFD; European Commission, 2008) represents the most comprehensive marine environmental legislation. This Directive aims to achieve Good Environmental Status (GES) by 2020 in the four European Regional Seas (Baltic, North Eastern Atlantic, Mediterranean and Black Sea). The MSFD requires that Member States assess ecosystem characteristics, pressures, and impacts with respect to 11 descriptors related to: biological diversity, non-indigenous species, commercial fish and shellfish, food-webs, eutrophication, seafloor integrity, hydrographic conditions, concentration of contaminants in the environment and in fish and other seafood consumed by humans, marine litter, and introduction of energy including underwater noise. Within these 11 descriptors, the European Commission (2010) then defines 29 criteria and 56 indicators necessary in evaluating environmental status.

The assessment of environmental status, while scientifically challenging (Stanley, 1995), simultaneously offers many opportunities for European marine research to support an ecosystem approach to environmental management, which EU Member States have agreed to implement (Borja et al., 2013). The European project DEVOTES (DEVelopment Of innovative Tools for understanding marine biodiversity and assessing GES, www.devotes-project.eu) was started in 2012 to facilitate MSFD implementation. This project considers these complex, inter-related scientific issues and management needs of the MSFD, as well as the challenges shared by the four regional seas identified within the MSFD. Its main objectives were:

- To improve understanding of the cumulative impacts of human activities on marine biodiversity and variation associated with climate, identifying the socio-economic and legislative barriers and bottlenecks that prevent achieving GES;
- To test indicators currently in use (European Commission, 2010) and develop new assessment options, particularly for biodiversity-related descriptors (i.e., D1. Biological diversity, D2. Non-indigenous species, D4. Food-webs, and D6. Seafloor integrity), at several ecological levels (species, habitat, ecosystems), and characterize and classify status of marine waters;
- To develop, test and validate innovative integrative modeling and cost-effective monitoring tools to strengthen understanding of ecosystem function and biodiversity changes in space and time associated with human impacts, including climatic influences.
- To propose and disseminate strategies and measures for adaptive management of ecosystems, including integrative and holistic tools to assess environmental status.

We therefore set an overall goal of better understanding the relationships between pressures from human activities and climate change, and their effects on marine ecosystems, including biological diversity, in order to support ecosystem-based management and attain GES of marine waters. Our harmonized approach to the four European regional seas tested and validated existing indicators, created new indicators when necessary, developed modeling tools for the assessment of biodiversity, tested new monitoring tools and established an integrative approach for assessing environmental status.

This overview describes how this research has contributed to advancing the state-of-the-art since 2012 in bridging the gap between science and policy in marine environmental status assessment. Specifically, this addresses elements such as human pressures, indicator development, model use, innovative monitoring, and integrative assessment tools), in order to achieve healthy and sustainable ocean use. Here we synthesize key responses to major environmental questions and the lessons learnt. This information will support managers and policy-makers in making decisions for improved management of ocean use.

WHY MUST WE UNDERSTAND IMPACTS OF HUMAN ACTIVITIES AT SEA?

State-of-the-Art

Marine environmental managers primarily aim to protect and maintain natural structure and functioning while simultaneously ensuring that ecosystems provide services, which in turn deliver benefits for society (Atkins et al., 2011; Elliott, 2011). In the management of human activities in the marine environment, it is axiomatic that a regulatory body (i.e., an environmental protection agency, natural conservation body, fisheries body, or marine licensing body), does not have to prove that an activity or its developer (the "user," "polluter"—those undertaking the activity, such as a dredging company, industrial plan, or wind farm operator) causes an adverse impact (Gray and Elliott, 2009). In contrast, the developer must prove they will not cause an impact, hence creating the scientific and statistical challenge of "proving the negative." A second key feature, "the precautionary principle" (PP), assumes a deleterious effect resulting from a given activity in the system unless proven otherwise (O'Riordan and Jordan, 1995). However, detractors criticize the vague definition of PP, and balancing scientific uncertainty and appropriate management measures remains a challenge (Steel, 2014).

The third key feature states that any developer wishing to use the marine system must obtain permission from a regulatory body, hence the importance of sufficient administrative bodies (Boyes and Elliott, 2014, 2015; Elliott, 2014); this encompasses the whole of marine governance, defined as the net result of policies, politics, legislation, and administration (Barnard and Elliott, 2015). The fourth feature, the "polluter pays principle," requires a developer to pay for the costs associated with that use: the licensing of the activity, the monitoring, remediation and mitigation of any damage to the system and, if necessary, compensation. The latter requires integrating natural and economic sciences to enable sustainability within and across generations and it may require developers to compensate affected users, the affected resource (e.g., restocking affected fish), or the affected environment (e.g., by creating new environment; Elliott et al., 2016). However, all of these central features relate to how users use an area of the sea (e.g., dredging, wind farm, fishing, etc.) but superimposing a wider suite of natural and human influences, such as climate change, on all of these activities (Elliott et al., 2015). This complexity demands, as the fifth feature, assessing the anthropogenic change or pressure in question (a "signal") against a background of inherent variability and natural change or wider influences, i.e., the changes emanating externally to the area being managed (the "noise"; Gray and Elliott, 2009; Elliott, 2011). Finally, a sixth key feature requires quantitative and legally defendable detection of such change with a direct feedback into management.

Progress beyond the State-of-the-Art

These key features require a defendable, holistic, underlying framework, accepted, and communicable to marine managers and wider users. That framework must link causes of potential and actual changes to the marine environment, the types of changes experienced and societal responses to mediating or removing the drivers of change or at least accepting change for the benefits provided. Even in the recent past, stakeholders frequently used the DPSIR (Drivers, Pressures, State change, Impact and Response) interlinking framework (e.g., Atkins et al., 2011; Smith et al., 2014), without clearly defining each element. Hence, the wide use of DPSIR model (Gari et al., 2015; Lewison et al., 2016; Patrício et al., 2016a) not only introduced many variants and perpetuated confusion but also made it not-fit-for-purpose in providing management guidance.

Previous studies document the evolution of the DPSIR approach (Smith et al., 2014, 2016), and here we summarize and focus on the evolution from DPSIR to the most recent derivative DAPSI(W)R(M) (Patrício et al., 2016a; Scharin et al., 2016; Burdon et al., in press). This modified approach adds Activities, and relates the Impact to human Welfare and the Responses to the use of Measures (the term preferred by EU Directives). Drivers describe underlying basic human needs, such as for food, security, space, and well-being, which require Activities (fishing, building wind farms, creating navigation routes). These activities then create Pressures, such as scraping the seabed with bottom trawls or building infrastructure that removes space. Pressures are the mechanisms that change the system, potentially causing concern. Those changes encompass both the natural system, including its structure and functioning (the "State change"; Strong et al., 2015), and the human system [the Impact (on human Welfare)]. The term Welfare is used *sensu stricto* to include economic welfare and human and societal well-being (Oxford English Dictionary).

Furthermore, all of the activities and external changes could potentially adversely affect that main aim (the protection of the social and ecological systems), and may thus be considered hazards. If these hazards damage parts of the socio-ecological system we value, they may be termed risks, thus providing a hazard and risk typology used in the DEVOTES project (Elliott et al., 2014). Smith et al. (2016) illustrated DPSIR, using fishing activity and the pressure of trawling from abrasion on the seabed and its impacts on particular components as an example. The challenges were addressed in moving from conceptual models to actual assessments including: assessment methodologies (interactive matrices, Bayesian Belief Networks, ecosystem modeling, the Bow Tie approach, assessment tools), data availability, confidence, scaling, cumulative impacts, and multiple simultaneous pressures, which more often occur in multi-use and multi-user areas (Smith et al., 2016).

Society and environmental managers need to know not only the current status of a marine system, but also whether it has been altered, the cause of that alteration, its significance, and what can be done to reverse that change. Therefore, this requirement creates the need to consider how Pressures result in State change, in the natural system, and a societally relevant Impact of sea use (including the assessment of cumulative pressures and impacts, as shown by Korpinen and Andersen, 2016); hence the need to consider not just Welfare (*sensu* DPSWR in Cooper, 2013) but the Impact (on human Welfare). This need explicitly includes an economic approach and a human health and well-being approach to human-induced changes. Furthermore, while that State change may often relate to the physico-chemical and ecological structure of the marine system, it increasingly requires users to consider the ecological functioning (Strong et al., 2015) especially given that many MSFD descriptors relate to functioning aspects. This "biodiversity-ecosystem functioning debate," regarding the effect of functioning on biodiversity and vice versa, is an important and developing field (Zeppilli et al., 2016).

The detection or prediction of changes to the natural state and impacts on human welfare require action to minimize, mitigate, compensate, remove, or even accept changes through societal Responses (the R in DPSIR). However, based on terminology used in the EU Directives, environmental managers now refer to those Responses as Measures [hence Responses -using Measures- in DAPSI(W)R(M); Scharin et al., 2016]. During the past decade, management has recognized the need to include all measures which therefore, as referred to as the Programme of Measures in the MSFD, should consider aspects of ecology, technology, economy, legislation, and administration. They should also satisfy societal, cultural and moral imperatives while communicating decisions to stakeholders; hence the so-called "10 tenets" for sustainable and successful marine management (Elliott, 2013; Barnard and Elliott, 2015).

The prevailing governance system provides a central control on adverse effects of human activities. The EU arguably represents the pre-eminent proponent of marine environmental legislation and other aspects of governance (Boyes and Elliott, 2014), but the complexity of the marine system, the need for transboundary action and the joint implementation of different systems have produced anomalies, confusion, and a need for an inter-governmental transboundary approach (Cavallo et al., 2016).

Most of the above framework relates to activities and pressures emanating from within a system such as a sea region, under management, for example the Baltic or North Seas (Andersen et al., 2015; Scharin et al., 2016). These may be termed endogenic managed pressures in which the causes and consequences in the region are managed (Elliott, 2011) and under legislative control (Boyes and Elliott, 2014). Exogenic unmanaged pressures (i.e., those aspects emanating from outside a managed system; for example global climate change Elliott et al., 2015) represent the major current challenge; environmental managers cannot control the causes but must respond to the consequences. Climate change offers a primary example, in which human impacts (e.g., ocean acidification, increase in alien species, sea-level rise, temperature regime change; Danovaro et al., 2013; Katsanevakis et al., 2014, 2016) add to internal pressures in an area. Climate change therefore shifts baselines, complicating evaluation change associated with internal activities in a region, but also potentially nullifying the use of quantitative indicators or at least requiring the target values of those indicators to be continually revised. A Member State not meeting legislative controls, such as directives, may therefore cite climate change as a modifying factor but one outside of its control (Elliott et al., 2015). Targets that cannot be reached due to changes caused by climate change effects are not manageable and need to be revised as a part of the 6 years management cycle.

Conclusions

Successful ocean use management relies on adequate and comprehensive monitoring, and identifying appropriate measurements of change. Management response requires a clear understanding of underlying causes and effects of change in the marine environment and their consequences. Hence, the use of conceptual models linking the marine drivers, activities, and pressures can provide that solid foundation to link to state changes, impacts on societal welfare, and the resulting management responses using programmes of measures. Similarly, management relies on the ability to predict and detect future responses of the system to changes with sufficient certainty; prediction requires conceptual, empirical, and deterministic models, whereas detection implies the presence of robust monitoring systems at appropriate spatial and temporal scales. However, the "paradox of environmental assessment" sets the backdrop for this framework whereby increasing national and European legislation (such as the MSFD) requires more understanding and better monitoring but monitoring organizations face reduced budgets (Borja and Elliott, 2013). Therefore, by expanding the concept of DPSIR into DAPSI(W)R(M), understanding the gaps and the Strengths, Weaknesses, Opportunities, and Threats in monitoring, and exploring how climate change could affect GES, DEVOTES has included human welfare in the modified approach, emphasizing the importance for future policy and management measures. Hence, an adequate assessment of marine status can only be achieved through fit-for-purpose monitoring based on sound scientific knowledge.

WHY DO WE NEED BETTER INDICATORS TO ASSESS THE STATUS?

State-of-the-Art

The multifaceted concept of biodiversity encompasses everything from the genetic composition of species to the organization of habitats and ecosystems (CBD, 1992). Despite the widely recognized need to maintain biodiversity, its many interpretations make difficult any comprehensive evaluation and therefore it is necessary to use indicators, or simplified measures, that reflect or synthesize the status of important aspects of ecosystem structure or function. Marine assessments depend upon indicators to detect and evaluate changes in environmental status driven by either natural or human pressures, often in the context of implementing management targets for environmental objectives and measures. Therefore, scientists and managers worldwide seek accurate and reliable indicators that represent all relevant aspects of marine biodiversity either as individual aspects or as surrogates (proxies) for series of changes (for example the use of the breeding health of piscivorous seabirds as a proxy for the whole marine trophic system).

Although, many nations worldwide recognize the need for an ecosystem approach to ocean management, the EU has led in developing specific metrics toward that objective. The European Commission (2010) Decision specifies criteria and methodological standards to evaluate environmental status of marine waters, based upon a set of 56 MSFD indicators. Some indicators used in the assessment of coastal ecosystems under the Water Framework Directive (WFD; European Commission, 2000; Birk et al., 2012) also apply to the MSFD assessment beyond the narrow coastal strip where MSFD and WFD overlap (Borja et al., 2010; Boyes et al., 2016). In practice, during the first phase of the MSFD implementation, EU Member States used different methodological approaches to determine and assess ecosystem status (European Commission, 2014; Palialexis et al., 2014). Data availability, regional specificities, and potentially different interpretations of the EU Commission Decision led to discrepancies within methodologies reported by Member States, increasing the potential for non-harmonized approaches to status determination. Managers require further guidance on criteria for "good" indicators, and assessment of status (Patrício et al., 2014), and such a plan is currently being developed by the EU and its Member States, ICES (International Council for the Exploration of the Sea), EEA (European Environment Agency), and RSCs (The Regional Sea Conventions). Concurrently, the RSCs are developing indicators for holistic marine assessments (e.g., HELCOM, 2013; OSPAR, 2015; UNEP, 2016).

Progress Beyond the State-of-the-Art

Overview of Existing Indicators and Gaps in Relation to MSFD Requirements

To support the MSFD process, we completed a comprehensive overview of existing MSFD biodiversity-related indicators (MSFD descriptors: D1—biological diversity, D2—non-indigenous species, D4—food-webs, and D6—seafloor integrity), identified gaps, and developed/tested new indicators to assess the status in the marine environment (Patrício et al., 2014).

We created an inventory of current MSFD biodiversity indicators, which includes over 600 entries, and developed complementary software (DEVOTool; www.devotes-project.eu/devotool) to help users navigate the metadata. The DEVOTool includes instructions for its use as well as a description of the database contents. Developing the inventory demonstrated that, despite many available marine biodiversity indicators, obvious gaps remain regarding some biotic components and criteria required for the MSFD implementation (Teixeira et al., 2014). Furthermore, information regarding the quality and confidence of the indicators is currently insufficient. Most available operational indicators target coastal and shelf ecosystems and cover WFD biological quality elements, such as macroinvertebrates, fish, phytoplankton, macroalgae, and seagrasses. Major current gaps include ecosystem level and genetic population level indicators, as well as indicators for microbes, pelagic and planktonic invertebrates, reptiles, ice-associated species, and communities, and deep-sea habitats. Most indicators lack regional targets or GES threshold values, and few measure confidence levels or demonstrably link to pressures. Thus, although current indicators may be regarded as operational in the way that they have been used in marine assessments, their applicability to fulfill the criteria of MSFD indicators and to comply with indicator quality criteria (Queirós et al., 2016) has not been assessed.

Development of New Indicators

We developed 16 new indicators and refined another 13 indicators (Berg et al., 2016; **Table 1**) to address gaps in MSFD implementation (Teixeira et al., 2014). These indicators mainly relate to the biodiversity-related Descriptors (D1, D2, D4, and D6), and cover the full range of biological components (i.e., from microbes to seabirds and marine mammals). In addition, we developed indicator quality criteria, which were used to evaluate these indicators (Queirós et al., 2016). For example, we developed four new indicators for microbes (bacteria and cyanobacteria), but their poor score on pressure responsiveness and the potential to set targets indicated a need for further development and validation (Berg et al., 2016). Some phytoplankton biomass indicators, such as chlorophyll-a concentration from satellite measurements, provide valuable assessments of pressures leading to eutrophication, but linking changes in diverse and rapidly fluctuating phytoplankton composition with impacts of nutrient loading has proved challenging (Camp et al., 2015; Carstensen et al., 2015).

Also indicators were developed to address the environmental impacts of invasive non-indigenous species in European regional seas (Minchin and Zaiko, 2013; Zaiko et al., 2014; Katsanevakis et al., 2016). Moreover, the project developed new food-web indicators focusing on primary and secondary producers, both for phytoplankton and fish. Of those, the novel food-web indicator "Phytoplankton community composition as a food-web indicator" was a highly-evaluated indicator and hence it is currently a candidate HELCOM core indicator for holistic ecosystem assessment. An indicator for systematic high-resolution habitat mapping and characterization scored high in the indicator-evaluation as it may be a proxy for many of the 56 MSFD indicators. We also recently developed and tested numerous promising indicators that capture effects of fishing on marine biodiversity, e.g., on the positive effects of fishing effort reduction on the increase of large fish indicator (Engelhard et al., 2015) and on the need of using biodiversity and conservation-based indicators complementarily to ecological indicators of fishing pressure to evaluate the overall impact of fishing on exploited marine ecosystems (Fu et al., 2015; Coll et al., 2016). Furthermore, a newly developed indicator based on DNA metabarcoding assesses genetic diversity of macroinvertebrates

TABLE 1 | Indicators developed or refined within DEVOTES project, in relation to some of the indicators proposed within the Marine Strategy Framework Directive (MSFD).

Marine Strategy Framework Directive requirements			DEVOTES indicators achievements	
Descriptors	**Indicator code**	**Indicator description**	**Indicators targeted**	**New or refined**
1. Biodiversity	1.1.2	Species: Distributional pattern within range	Distribution of herbivorous waterfowl in relation to eelgrass biomass distribution	New
	1.2.1	Species: Population abundance and/or biomass	Microbe biodiversity and indicator species	New
	1.3.1	Species: Population demographic characteristics	Microbe biodiversity and indicator species	New
	1.4.1	Habitats: Distributional range	Lower depth distribution limit of macrophyte species	Refined
	1.4.2	Habitats: Distributional pattern	Distribution of herbivorous waterfowl in relation to eelgrass biomass distribution	New
	1.5.1	Habitats: Habitat area	High resolution habitat characterization	New
	1.6.2	Habitats: Condition of the typical species and communities	Production of phytoplankton	New
			Phytoplankton community composition as a food web indicator	New
			Phytoplankton community composition based on food quality traits as an early warning indicator for food web effects on higher trophic levels	New
			Phytoplankton taxonomic diversity (Shannon95)	Refined
			Phytoplankton taxonomic evenness	New
			Seasonal progression of phytoplankton functional groups	Refined
			Spring diatom/dinoflagellate biomass ratio (Black Sea)	New
			Biomass of copepods	Refined
			Mesozooplankton biomass	Refined
	1.6.3	Habitats: Physical, hydrological and chemical conditions	High resolution habitat characterization	New
2. Non-indigenous species	2.1.1	Trends in abundance, temporal occurrence and spatial distribution	Abundance and distribution range of established Non-Indigenous Species	Refined
			Trends in the arrival of new non-indigenous species	New
			Trends in the arrival of Non-Indigenous Species by pathway of entry	Refined
	2.2.2	Impacts of non-indigenous invasive species	Cumulative impact index of Invasive Alien Species	New
3. Commercial species	3.3.2	Mean maximum length	Mean maximum length of demersal fish and elasmobranchs	New
4. Food-webs	4.2.1	Large fish	Size composition in fish communities (Typical length)	New
			Large fish indicator	New
	4.3.1	Abundance trends of functionally important selected groups/species	Share of cyanobacteria from total phytoplankton biomass as an early warning indicator for food web effects on zooplankton	New
			Production of phytoplankton	New
			Phytoplankton community composition as a food web indicator	New
			Phytoplankton community composition based on food quality traits as an early warning indicator for food web effects on higher trophic levels	New
			Biomass of copepods	Refined
			Mesozooplankton biomass	Refined
			Distribution of herbivorous waterfowl in relation to eelgrass biomass distribution	New

(Continued)

TABLE 1 | Continued

Marine Strategy Framework Directive requirements			DEVOTES indicators achievements	
Descriptors	**Indicator code**	**Indicator description**	**Indicators targeted**	**New or refined**
5. Eutrophication	5.2.1	Chlorophyll concentration in the water column	Surface Chlorophyll-a concentration from satellite measurements	Refined
	5.2.4	Species shift in floristic composition such as diatom to flagellate ratio, benthic to pelagic shifts, as well as bloom events of nuisance/toxic algal blooms caused by human activities	Biomass of N2-fixing cyanobacteria as an indicator for nitrogen load originating from N2-fixing cyanobacteria	New
			Diatom/Dinoflagellate index	Refined
			Spring diatom/dinoflagellate biomass ratio (Black Sea)	New
	5.3.1	Abundance of perennial seaweeds and seagrasses adversely impacted by decrease in water transparency	Lower depth distribution limit of macrophyte species	Refined
6. Seafloor integrity	6.1.2	Extent of the seabed significantly affected by human activities for the different substrate types	High resolution habitat characterization	New
	6.2.1	Presence of particular sensitive and/or tolerant species	Genetic based benthic microbial community condition and functionality assessment	New
			AZTI's Marine Biotic Index (AMBI)	Refined
			Genetic based macrobenthic community condition and functionality assessment	New
	6.2.2	Multi-metric indices assessing benthic community condition and functionality	Multivariate AZTI Marine Biotic Index	Refined
			Benthic quality index	Refined

Note the potential application of some indicators in assessing various MSFD indicators.

and microorganisms (Aylagas et al., 2014, 2016; Carugati et al., 2015; Dell'Anno et al., 2015).

We also applied Signal Detection Theory (SDT) to assess the accuracy, sensitivity and specificity of refined benthic indicators (such as the Benthic Quality Index—BQI) and their response to eutrophication. In general, we found SDT to be a robust and scientifically sound strategy for setting threshold values for indicators (Chuševė et al., 2016). Finally, we introduced a new approach to set indicator targets in relation to ecosystem resilience (i.e., the ability to recover rapidly and predictably from pressures) and to select indicators and their target ranges (Rossberg et al., 2017). This approach is a specific, quantitative interpretation of the concepts of GES and sustainable use in terms of indicators and associated targets. Importantly, it distinguishes between current and future uses to satisfy societal needs and preferences.

Conclusions

Increasing legal challenges of marine and coastal management, both to the EU Member State implementation of Directives and industry compliance with national laws, which hinge upon detecting and demonstrating marine environmental change (Elliott et al., 2015), increases the need for scientifically defensible indicators. Those indicators must be comprehensive, either in covering all relevant aspects of the marine system or as conceptually defensible surrogates that represent a well-defined and well-accepted causal link (e.g., the health of breeding populations of top seabird and fish predators being dependent on the health of seabed populations).

We tested and refined 13 available biodiversity indicators, developed 16 new options for assessment, particularly for biological descriptors (considering species, habitat and ecosystem levels), identified gaps for future research, developed indicator performance criteria, and provided a user-friendly tool to select and rank indicators (**Table 1**). These publicly-available contributions (Berg et al., 2016), support the second phase of the MSFD implementation and assist marine management in Europe and elsewhere.

WHY MODELS ARE NECESSARY IN MARINE STUDIES AND ASSESSMENT?

State-of-the-Art

Understanding how changes in biodiversity link to food-web functioning, anthropogenic pressures, and climate changes requires novel, integrative modeling tools. Similarly, scaling determining change from small to large areas and from the present to future, also requires such modeling approaches. Once validated, modeling tools can elucidate expected risks and rewards for a range of management options, aimed at achieving or maintaining GES. The evidence base from such scenario testing thus provides a suitable platform to enable informed decision-making. Prior to 2012, the proposals for using models in the MSFD implementation were very limited (Cardoso et al., 2010) but now, in the context of using models in assessments, Pinnegar et al. (2014) for example have demonstrated the value of food-web models in assessing potential responses of ecosystems to invasions.

Progress beyond State-of-the-Art

We assessed the capabilities of state-of-art models to provide information about current and candidate indicators outlined in the MSFD, particularly on biological diversity, food-webs, non-indigenous species, and seafloor integrity descriptors (Piroddi et al., 2015; Tedesco et al., 2016). We demonstrated that models could explain food-webs and biological diversity, but poorly-addressed non-indigenous (alien) species, habitats and seafloor integrity (Lynam et al., 2016).

Habitats and Non-Indigenous (Alien) Species

In order to address the key gap related to non-indigenous (alien) species, we developed a method to model the vulnerability of areas to invasions, using the Mediterranean Sea as a case study (Katsanevakis et al., 2016). This conservative additive model accounts for the Cumulative IMPacts of invasive ALien species (CIMPAL index) on marine ecosystems. It estimates cumulative impact scores based on distributions of invasive species and ecosystems, considering both the reported magnitude of ecological impacts and the strength of such evidence.

Theory and New Approaches to Model Ecosystem Function

The theory supporting advanced modeling of food-webs and biodiversity was extended (Rossberg, 2013; James et al., 2015). Through different projects, including DEVOTES, Fung et al. (2015) used this theory to explore links between Biodiversity-Ecosystem Functioning (BEF) in marine ecosystems to fill in a key knowledge gap. Strong et al. (2015), furthermore, showed the importance and potential of such functional indicators. The BEF relationship can change (Mora et al., 2014; Fung et al., 2015), but the protection of fish from predation provided the mechanism in this case, and BEF relationships depended upon species richness and fishing impacts. Previous studies by Danovaro et al. (2008) revealed that the BEF relationships can be exponential and thus extremely sensitive to changes in environmental conditions determining a biodiversity loss. Nagelkerke and Rossberg (2014) also developed a theoretical understanding whereby resource and consumer traits predict trophic space, such that empirical data can be used to determine trophic traits related to food-web functioning (James et al., 2015).

New modeling approaches using mass-balanced models were also developed to identify ecosystem structure, function (including Ecological Network Analyses) and reaction to disturbance (Lassalle et al., 2013, 2014a,b; Niquil et al., 2014; Chaalali et al., 2015; Guesnet et al., 2015).

Habitats and Function

To further understand the role of habitat in regulating function in marine food-webs, and thus link to other descriptors, such as seafloor integrity and biological diversity, we studied marine habitats at local [i.e., Basque coast, Galparsoro et al. (2015); Eastern Aegean Sea, Lynam et al. (2015b); Western Adriatic deep-sea, Zeppilli et al. (2016)], sub-regional (i.e., North Sea, Stephens and Diesing, 2015; van Leeuwen et al., 2015), and regional (i.e., Mediterranean, Katsanevakis et al., 2016) scales. For example, we developed a process-driven characterization of sedimentary habitats for the Basque continental shelf and demonstrated that species richness decreases rapidly with increased sediment resuspension (Galparsoro et al., 2013). Habitat modeling of elasmobranchs in the southern North Sea demonstrated the extirpation of some species such as common skate over time (Sguotti et al., 2016). Modeling spatial distribution of three common seabird species in the southern North Sea demonstrated the importance of habitat type and availability fish prey to seabird distributions (see Lynam et al., 2015a). Additionally, we demonstrated in Stephens and Diesing (2015) the feasibility of predicting substratum composition spatially across a large swath of seabed (North Sea) using legacy grain-size data and environmental predictors. We also demonstrated the suitability of such a quantitative prediction for further analyses of habitat suitability compared to traditional grid cell categorization (Stephens and Diesing, 2015).

We applied Benthic Traits Analysis (Alves et al., 2014; van der Linden et al., 2016a; Van der Linden et al., 2016b) specifically to understand benthic community function in relation to habitat. This analysis identified typological groups of benthic macroinvertebrates in the North Sea, based on response and effect traits, as potential ecological indicators for MSFD Descriptors 1 (Biological Diversity) and 6 (Seafloor integrity; Veríssimo et al., 2015). The creation and analysis of large data set on population genetics in species groups with different dispersal abilities linked genetic variation to constraints in movement within benthic habitats in macroinvertebrates. This finding appears consistent with a “neutral theory” explanation for marine biodiversity spatial patterns (Chust et al., 2013, 2016).

A major challenge in marine management and assessment relates to the ability to link the physico-chemical and ecological systems. For example, for pelagic habitats, we identified distinct physical regimes in the North Sea based on density stratification characteristics, and modeling identified five hydrodynamic regimes (van Leeuwen et al., 2015). These findings are valuable to support assessment at a sub-divisional scale within MSFD subregions. Effective marine management must consider these regimes and their likely biological interactions. These zones form the basis for the OSPAR biodiversity (pelagic habitat) assessment based on lifeforms, together with considering oxygen and eutrophication when assessing primary production for food webs.

Scenario Testing to Inform Management Decisions

Our research demonstrated that fisheries management may enhance biological diversity (such as the size-structure of the fish and elasmobranch community) but potentially produce unintended consequences for other ecosystem components (Lynam and Mackinson, 2015). For example, decreases in bentho-piscivores component. However, the system may nonetheless sustain economic yields with minimal risk of stock collapse (Lynam et al., 2015b) if managed through an ecosystem approach. In the long term, climate change may shift baselines for indicators (Lynam et al., 2015b) and so assessments of GES should recognize these effects (Elliott et al., 2015).

Conclusions

Marine research and assessment require modeling studies that can support the use of indicators in fully encompass the functional linkages between ecosystem components and overwhelming pressures on the marine environment, such as climate change and ocean acidification. Such modeling provides the evidence for setting realistic targets and thus supporting better long-term marine planning. We used case studies to illustrate that modeling can assist in MSFD implementation, contributing to each step of the assessment and management cycle. Modeling can help to develop and refine novel indicators to support indicator-based assessment of GES. Modeling can incorporate indicator trends and responses, incorporating prevailing climatic conditions and anthropogenic pressures and, in this way, support the review of objectives, targets and indicators. Moreover, modeling can both inform adaptive monitoring programmes and be used in scenario testing to inform management decisions.

MONITORING NETWORKS IN EUROPEAN REGIONAL SEAS: IS TRADITIONAL MONITORING SUFFICIENT TO ASSESS THE STATUS OF MARINE ECOSYSTEMS?

State-of-the-Art

Methods traditionally used in marine monitoring to investigate spatial and temporal variation in abiotic and biotic variables are time-consuming, costly and often limited in resolution (de Jonge et al., 2006; Borja and Elliott, 2013; Carstensen, 2014; Fraschetti et al., 2016). These constraints can severely limit our capacity to detect spatial and temporal changes in marine environmental health. In addition, most countries lack the tools to expand marine monitoring to the deep sea (Ramirez-Llodra et al., 2011), severely constraining the expected implementation of the MSFD in the open ocean and deep sea (Zeppilli et al., 2016). Moreover, marine monitoring methods currently limit analyses of some descriptors. For example, detecting cryptic and/or alien species (including those causing harmful algal blooms) will benefit from molecular approaches (Bourlat et al., 2013).

Activities that smoother, abrade or permanently-remove seabed habitat represent the greatest threats to seafloor integrity (Rice et al., 2012). Previous studies used benthic faunal analysis to indicate general seafloor integrity (Pearson and Rosenberg, 1978), drawing on an extensive catalog of methods and approaches for such a fundamental change (Gray and Elliott, 2009), but increasingly together with various visual assessment tools (Solan et al., 2003). Specific benthic faunal indicators exist for trawl abrasion (Jorgensen et al., 2016) but deriving these indicators is time-consuming and expensive to implement. Video inspection of seafloor smothering using Remotely Operated Vehicles (ROV), such as from seabed drilling activities, can visually map the environmental footprint (Gates and Jones, 2012), but we lack data to validate the uncertainty of the method compared to conventional biological sample collection.

The implementation of the assessments of marine environmental status required by the MSFD thus requires development and/or testing of innovative monitoring systems. Despite creating recent methodologies/technologies in DEVOTES, these are not yet used in routine monitoring of the MSFD descriptors. We encourage this through our summary analysis encompassing a catalog of monitoring networks and a wide array of potential tools, including: (i) molecular approaches (e.g., barcoding and metagenomic tools), (ii) remote sensing/acoustic methods, and (iii) *in situ* monitoring techniques.

Progress beyond the State-of-the-Art

We have produced a catalog with the biodiversity monitoring networks, currently available in European Seas, with the aim to: (i) present a critical overview of the monitoring activities in Europe (i.e., the amount and reason for ongoing monitoring, whether it fulfills its objectives and to what pressures it is links), (ii) identify areas where no monitoring occurs, and (iii) recommend the further development and improvements for optimizing marine biodiversity monitoring in the context of the MSFD. Since the publication of the catalog (Patrício et al., 2014), new material has been added so that it currently identifies 865 monitoring activities corresponding to 298 monitoring programmes. A gap and SWOT (Strengths, Weaknesses, Opportunities, and Threat) analysis of the catalog (Patrício et al., 2016b), highlights uneven distributions of monitoring across regional seas (i.e., more monitoring activities in the North Eastern Atlantic and Mediterranean). Specifically, we note uneven monitoring effort between descriptors (e.g., more monitoring for Descriptor 1 on Biological diversity and Descriptor 4 on Food webs), between biological components (e.g., monitoring emphasis on fish and phytoplankton) and between pressures (e.g., high level of monitoring of organic matter enrichment across all regional sea). In addition, we consider whether monitoring networks are fit-for-purpose or sufficient for adequate implementation of the MSFD within the context of the need for better coordination, harmonization of methodologies, and cost-effectiveness considerations (Patrício et al., 2016b). This allowed us to explore different innovative monitoring approaches. Below we discuss these new approaches in terms of their potential applications to some of the 11 descriptors of the MSFD investigated by DEVOTES, in order to evaluate their broader applicability to future marine environmental monitoring.

Descriptors 1 (Biological Diversity) and 2 (Non-indigenous Species)

Future monitoring is increasingly likely to use molecular tools to complement classical taxonomic techniques in providing timely and inexpensive results (Bourlat et al., 2013). Classical biodiversity assessment is time-consuming and requires diverse taxonomic expertise. Metabarcoding could expedite biodiversity assessment, especially for microscopic organisms (either algae or animals) for which morphological identification is difficult (Carugati et al., 2015). For example, Dell'Anno et al. (2015) provided the first comparison of different DNA extraction procedures and their suitability for sequencing analyses of 18S rDNA of marine nematodes. They subsequently analyzed intra-genomic variation in 18S rRNA gene repeats and reported that morphological identification of deep-sea nematodes matches the results obtained by metabarcoding analysis only at the order-family level. These results illustrate the importance of metabarcoding for exploring the diversity of benthic metazoans, but currently available databases have a limited coverage in quantifying the species encountered. Metabarcoding studies should therefore carefully consider these limitations in quantitative ecological research and monitoring programmes of marine biodiversity (Aylagas et al., 2016).

The routine use of microarrays for rapid detection of specific phytoplankton taxa, and particularly the presence of harmful algal blooms, requires further development to increase reliability and reduce associated time and expense. Nonetheless, monitoring strategies should include different molecular approaches [e.g., quantitative, *in situ* Polymerase Chain Reaction (PCR)] as these approaches offer far greater sensitivity to detect the presence, for example, of pathogenic bacteria compared to traditional approaches.

In addition to the above molecular tools, comparing biodiversity across different habitats and seas represents a critically important aspect of marine biodiversity monitoring, which metabarcoding can address. For example, in order to use metabarcoding to investigate the benthic biodiversity colonizing identical structures in different habitats, we deployed and later recovered Autonomous Reef Monitoring Structures (ARMS), initially developed by NOAA for coral reefs, after 12 months on hard bottoms at shallow depths at three sites (triplicates) within different regional seas (Baltic Sea, English Channel in the NE Atlantic, Adriatic Sea, Black Sea, and Red Sea). This highly reproducible approach allows a standardized comparison of colonizing biodiversity in different systems.

In parallel, molecular tools allowed us to identify aspects of biodiversity that classical tools could not, such as identifying microbial assemblages as indicators of biodiversity (Caruso et al., 2016), monitoring picoplankton (Ferrera et al., 2016), an early detection of invasive species (Ardura et al., 2015; Zaiko et al., 2015a,b), a census of meiofauna (Carugati et al., 2015), identifying functional gene diversity and plankton phylogeny (Reñé et al., 2013, 2015; Ferrera et al., 2015), revealing benthic eukaryotic diversity (Pearman et al., 2016a,b), or assessing the status of benthic macroinvertebrates (Aylagas et al., 2014).

The MSFD recognizes spatial changes in species and population distributions as key indicators. Numerous DEVOTES studies demonstrated the value of combining seabed geological information with biological variables (e.g., Galparsoro et al., 2013, 2014). However, whilst multiple needs drive the collection of such geological data (e.g., safety of navigation, renewable energy infrastructure, planning), mapping the entire marine area will require considerable time (although perhaps less than a decade with existing capabilities). Despite this potential, even after a comprehensive baseline survey, further monitoring for change will always be necessary. Existing monitoring programmes have enabled collection of high-resolution multibeam sonar data over a large area and extrapolation of these properties across 100,000 km^2 in the western English Channel. Only by addressing and interrogating environmental variables at scales and a resolution relevant to the biota will we understand the context of local ecosystem change and status.

Descriptor 3 (Commercial Fish Species and Shellfish)

At present, other than acoustic surveys that lack taxonomic resolution and exceed the science capability of developing nations, we lack novel approaches to replace traditional surveys and stock recruitment assessment in fish population studies. However, emerging molecular tools can identify connectivity among fish populations and help elucidate the role of connectivity in maintenance of fish stocks.

Descriptor 4 (Food-Webs)

Researchers can now cost-effectively monitor the functioning at the base of the food-web (i.e., primary and secondary production) using ferrybox systems [such as the Continuous Automated Litter and Plankton Sampler -CALPS-, developed on the RV Endeavor CONISMA, 2013] on research vessels and ships of opportunity. The zooplankton data collected by CALPS identifies broad geographic patterns in abundance and diversity and can be integrated within existing multidisciplinary surveys at minimal extra cost. As another example, semi-automated classification of zooplankton samples usefully provided data for a range of food web related indicators even in the northern Baltic Sea, where the generally small-bodied zooplankton is difficult to be classified using semi-automated methods (Uusitalo et al., 2016a). The OSPAR-led EU project "Applying an ecosystem approach to (sub) regional habitat assessments" (EcapRHA, www.ospar.org/work-areas/bdc/ecaprha) has further investigated this approach. Monitoring of phytoplankton community composition (i.e., ratio between diatoms and flagellates) by a combination of remote sensing, microscopy, and bio-optical methods can clarify food-web effects on higher trophic levels (Goela et al., 2015).

Descriptor 5 (Eutrophication)

Current instruments that can analyze chlorophyll-a from *in situ* sampling can ground-truth satellite image analysis for monitoring of phyto-pigments concentrations in surface waters (Cristina et al., 2014, 2016) or assess aquaculture impacts (Mirto et al., 2010, 2014; Luna et al., 2013; Bengil and Bizsel, 2014). In addition, pigment color analysis (particularly *in situ* flow cytometry) can provide insights on phytoplankton biodiversity (Goela et al., 2015), estimate and calculate time series of annual gross primary production, and support MSFD implementation (Cristina et al., 2015). We also investigated the influence of benthic trophic state on meiofaunal biodiversity and found that the benthic trophic status based on organic matter variables is not sufficient to provide a sound assessment of the environmental quality in marine coastal ecosystems. However, the integration of the meiofaunal variable allows providing robust assessments of the marine environmental status (Bianchelli et al., 2016).

Descriptor 8 (Contaminants)

Andrade et al. (accepted) developed a high frequency non-invasive (HFNI) bio-sensor as a potential tool for marine monitoring which uses the biorhythmic gaping behavior of clams (such as the Icelandic scallop *Chlamys islandica* and the Pacific oyster *Crassostrea gigas*) in response to environmental cues such as day length. These innovative microsensors measure the distance between the valves of bivalves held in underwater baskets at strategic locations, and can operate unattended for several years. Measurements every 1.6 s are telemetered from the field to the laboratory and further transferred to a "big-data" storage system for analysis. Minimal operational costs and online, real-time data availability offer major advantages of the system once installed.

Beyond biorhythm research (including growth and spawning behavior) in relation to climatic factors, the method has potential for monitoring marine contamination. Exposure to stressors such as sudden changes in water quality, temperature increases (e.g., around power plants), toxic algal blooms, or a plume of water-borne contaminants, interrupts otherwise regular gaping behavior. The automated, real-time detection could provide an early-warning system, with potential applications including monitoring of water quality at swimming beaches, harbors, petroleum installations (produced water and unintentional spillages), and aquaculture sites. This "talking clam" method can improve cost-efficiency by alerting users to periods of potential risk, narrowing the need for more labor-intensive physical sampling, as long as it is assumed that normal gaping behavior reflects good water quality status.

Conclusions

As indicated above, we currently face a "paradox of environmental assessment"—with increasing monitoring requirements set against a backdrop of decreasing budgets. This paradox ensures the need for more cost-efficient and effective monitoring, and may eventually produce cheaper traditional monitoring, especially where monitoring requirements span large areas, as in the MSFD. The paradox requires wide-scale and rapid surveillance techniques, including innovative tools such as genomic approaches, remote sensing and acoustic sensors.

WHY DO WE NEED AN INTEGRATIVE ASSESSMENT OF STATUS?

State-of-the-Art

The European Commission (2010) identified 56 indicators to consider when evaluating environmental status, but at least an order of magnitude more indicators already exist (Berg et al., 2015). Despite this, many of these indicators are variants on similar themes and hence measure related attributes, and are often geographical derivations, for example the health of seabed communities. The relevance and availability of indicators vary substantially among regional seas and their subdivisions; however, the MSFD provides no guidance on integration principles, despite multiple approaches to aggregating indicators whose selection may produce highly diverging results (Borja et al., 2014). These choices challenge the scientific community to develop harmonized approaches for integrating these indicators to compare across different assessment areas.

The Ocean Health Index (OHI; Halpern et al., 2012) was developed to assess the consequences of human impacts as well as societal benefits by calculating a weighted average of scores for pressure, status and resilience goals in different areas globally. Borja et al. (2011) were the first to address specifically the challenges of the MSFD, using weighting averaging principles for integrating indicator information. The MARMONI (Innovative approaches for MARine biodiversity MONItoring and assessment of conservation status of nature values in the Baltic Sea) assessment tool (Martin et al., 2015) then used an aggregation principle based on the hierarchical structure laid out by the European Commission (2010), rather than using aggregation approaches based on the structures of marine ecosystems. Nevertheless, all assessment methods standardize indicators to a common scale prior to aggregation (Borja et al., 2016). This standardization relies upon defining of targets or reference states, which MSFD describes as targets for GES (Borja et al., 2013). The OHI uses the relative deviation from a reference state, whereas the MARMONI tool uses a binary scoring system to determine whether GES has been achieved (score of 100) or not (score of 0). However, these standardization approaches do not always achieve translating indicator values to a common scale. A relative deviation from a reference state of 50% could indicate a minor human disturbance for one indicator but a major human disturbance for another. Similarly, a binary standardization approach does not differentiate between whether minimal attainment or high status level of GES was achieved.

Progress beyond the State-of-the-Art

We developed and released software for NEAT (Nested Environmental status Assessment Tool; freely available at: www.devotes-project.eu/neat), to overcome some of the deficiencies of current integrated assessment tools (e.g., aggregation of multiple indicators at multiple temporal and spatial scales; absence of uncertainty determination, etc.) NEAT is loosely based on previous tools (Andersen et al., 2014, 2016) and translates indicator values to a common scale ranging from 0 (worst possible status) to 1 (best possible status), with 0.6 defining GES or the good-moderate boundary according to the WFD. Similarly, NEAT also allows users to set boundaries representing high-good status (value of 0.8), moderate-poor status (value of 0.4), and poor-bad status (value of 0.2). It also employs stepwise linear interpolation between these fixed points to produce transformations with a high degree of flexibility spanning the entire scale (0–1) and in which 0.6 always represent GES. In comparison with the OHI and MARMONI tool, this transformation produces a more comparable scale for integrating standardized indicator values. NEAT also employs weighted averaging of standardized indicators, but bases averaging on ecosystem features to represent the whole ecosystem. The approach primarily divides the entire ecosystem into multiple Spatial Assessment Units (SAU) that are nested to define a hierarchy of SAUs. Habitat information and relevant indicators according to organism groups are used to describe the environmental status which the given habitat may enter at different levels of the hierarchy, depending on the spatial representativity of the indicator and organism. First, averaging aggregate indicators at the organism level to produce a more even representation of relevant organism groups, i.e., to avoid an assessment biased by many indicators for the same organism group, before aggregating across habitats and SAU (Clark et al., 2011). Spatial information of the different SAUs, if provided, is used for weighting and habitats can be prioritized to weight complex habitats such as vegetated sea bottoms more heavily than deep, muddy sediments. In addition, NEAT indicators are associated with the different MSFD descriptors, supporting assessments based on various descriptor combinations (essentially from one to all).

Application of NEAT to 10 case studies across European marine waters with very different challenges, environmental conditions, and scales (Uusitalo et al., 2016b) highlights its flexibility adapting to these very different cases. This also highlighted the need for careful evaluation of the indicator set, their GES boundaries, and the selection of the SAUs, all of which can increase the accuracy of the GES assessment.

Finally, NEAT includes an uncertainty assessment at all levels of integration based on the propagation of errors (uncertainties) associated with the provided indicator information (Uusitalo et al., 2015). Therefore, assessing the confidence in the integrated assessment requires including an indicator value with an estimate of the standard error of that indicator value. Noting that few studies report or even determine the standard error of an indicator value, Carstensen and Lindegarth (2016) provide a framework for quantifying indicator uncertainty to enable such calculations. Knowing the distributions of the indicator estimates enables the calculation of the distribution of the standardized indicators as well as their aggregated values.

Conclusions

A true ecosystem approach for ocean use management requires an integrative assessment of marine water status. In this way, NEAT provides a second-generation, integrated assessment tool that builds on the hierarchical structure of marine ecosystems and the organisms inhabiting different compartments within this structure, thereby improving upon previous tools. Such a hierarchical approach allows users to interrogate the results to understand the reasons for the failure or success at achieving GES. However, the integrated assessment is only as good as indicator information allows, and missing or omitting information on specific groups (e.g., biological components or descriptors relevant to the assessed area) can bias the assessment results. Therefore, managers should produce guidelines stipulating indicator minimum requirements [e.g., type, coverage (ecosystem components, area, etc.), number] and the integrated assessment tool should clearly indicate if there is non-compliance with such guidelines. Moreover, because NEAT includes a comprehensive uncertainty assessment, researchers should incorporate this information as part of their interpretation of outcomes and decision support, thus needing guidelines for confidence levels of decisions.

In conclusion, environmental managers must assess the status of marine waters, not only to comply with current legislation (i.e., MSFD, WFD), but also to determine how far from targets marine ecosystems may be. Such information will allow managers to make informed decisions on sustainable resource use and the adequate restoration of degraded systems.

WHAT ECONOMIC AND SOCIAL DIMENSIONS AFFECT MARINE MANAGEMENT?

State-of-the-Art

Inevitably, new legislative framework directives bring about unforeseen challenges to the different stakeholders who need to be involved in their implementation, particularly when first applied. Managers already apply the MSFD, which is itself complex, to complex, heterogeneous, and dynamic environments. Furthermore, initiation of the MSFD coincided with a period of a growing, global economic crisis. The numerous objectives can potentially conflict with one another from the perspective of different government departments within the Member States and also between Member States sharing a regional sea. The MSFD legal status and implementation deadlines demand that scientists and decision makers ensure a collaborative and multidisciplinary approach to deliver multi-sectoral objectives that test the abilities of existing institutions. The rapid identification of the issues, and the problems that they can create, can help those responsible for MSFD implementation to consider best how to address such issues and ensure that the MSFD can provide the intended sustainable environmental benefits.

The introduction of complex and integrative environmental legislation such as the MSFD also inevitably incurs additional costs, such as establishing new monitoring and improving existing monitoring of multiple indicators across European seas. This demand can be economically challenging. Policy makers and regulators in all EU countries are obliged to manage their resources carefully and hence they will seek to comply with the MSFD in the most cost-effective way. Yet they have many choices on which types of monitoring to apply as they select the approaches that best comply with the legislative needs within the limits of their budgets (Veidemane and Pakalniete, 2015). Although the MSFD does not require consideration of the socio-economic aspects of monitoring, Borja and Elliott (2013) noted that limited financial resources represent the most significant threat to ensuring adequate monitoring.

Furthermore, the law requires that EU countries determine whether they need new management measures and monitoring schemes to enable them to achieve GES and, if so, to implement them. Here, the socio-economic analysis of the use of marine waters, the cost of present-date degradation of the marine environment, and the cost-benefit analysis of implementing monitoring and new management measures required under the MSFD could motivate Member States to achieve GES. However, whilst a dominant tool of all governments, economic analysis approaches to achieve such analyses specifically for the MSFD in relation to the marine environment and its management were not developed at the start of the MSFD process.

Progress Beyond the State-of-the-Art

Barriers to Achieving Good Environmental Status

A comprehensive review of the documented barriers to achieving GES indicated that Member States have encountered and reported legislative, governance, and socio-economic barriers during this first phase of implementing the MSFD (Boyes et al., 2015, 2016). Barriers include ambiguity in the text of the Directive resulting in different interpretations by Member States, creating uncertainty, and different levels of conformity and governance complications. For example, GES [Article 3(5)] is neither well defined nor quantitatively described (Boyes et al., 2016), not easily understandable, and requires specific guidance to achieve common understanding and to enable coherent practices between the Member States and across regional seas. The next revision of the European Commission (2010) Decision regarding MSFD implementation will provide more guidance on GES definition (for example the operational definition proposed by DEVOTES; Borja et al., 2013), and thus the input from different stakeholders, including the scientific community, will be extremely important. The effectiveness with which MSFD can achieve GES partially relates to the success of other EU legislation [e.g., the WFD, the reformed Common Fisheries Policy (CFP), Maritime Spatial Planning Directive (MSP), Integrated Maritime Policy (IMP)], acknowledging the ambiguity of the role and contribution of each individual piece of legislation. Despite limited reference to specific policies in the MSFD, it provides a framework that can incorporate earlier and future legislation to ensure that legislation provides spatially and temporally complete coverage for the protection of marine environment. The MSFD article 6 is quite clear on the purpose and role of RSC: "*... Member States shall, where practical and appropriate, use existing regional institutional cooperation structures, including those under Regional Sea Conventions...*" and "*...Member States shall, as far as possible, build upon relevant existing programmes and activities developed in the framework of structures stemming from international agreements such as Regional Sea Conventions....*" However, in the absence of clear guidance on how this objective should be implemented or the actual competence of the RSCs, Member States have not adopted the regional coordination and integration to achieve MSFD objectives. Boyes et al. (2015, 2016) offer recommendations to address these legislative and governance barriers, such as "*continued clarification and harmonization of the definitions and methodologies within and between Member States and the different RSCs*." The aims of other directives should be consistently included in considerations for GES together with clear reference to MSFD and other existing, forthcoming and amended directives. Systematic use of standards that already used within other EU legislation must be applied as minimum requirements. Implementation of the MSP Directive particularly provides measures that will support delivery of the goals of MSFD by facilitating a balance with blue growth objectives (Boyes and Elliott, 2014; Boyes et al., 2016). The RSC must have a mandate supported by their contracting parties in order to ensure that the measures implemented in EU countries are supported and complemented by respective measures also in non-EU countries. Achieving RSC aims requires continuous cooperation in regional seas between EU-Member and non-Member States in the context of RSCs (Cavallo et al., 2016).

Socio-economic barriers include a lack of appropriate biological, environmental, and socio-economic data, a limited application of the ecosystem-based approach and of economic impact analyses by Member States. Effective use of the findings of EU funded projects and pilot projects (involving both non-EU countries and Member States) can both boost the evidence and knowledge required. It can also provide a vehicle to improve and support regional coordination and encourage the coherent implementation of the MSFD in regional sea areas, and ensure engaging non-EU countries in programmes that enable measures to achieve true regional GES.

Discussions with stakeholders showed that often public and stakeholder consultations on the programmes of measures were only open for limited periods of 1–2 months, and stakeholders in most Member States, particularly NGOs, felt that they were not sufficiently involved in the MSFD process, with only limited integration of their feedback (Boyes et al., 2015). In recognizing the complexity of marine ecosystems, the existence of multiple stakeholders with imperfect and impartial knowledge, as well as resource constraints, we developed a workshop approach "to engage and share different perspectives, and develop models of the system under consideration that are seen to be valid and useful aids to decision making" (Boyes et al., 2015). This multi-stakeholder workshop based modeling approach, which focused on Causal Loop Diagrams (CLD) to describe and understand the case site, was developed and then trialed in a case study site in England (Boyes et al., 2015). Managers should consider this approach, which effectively engaged stakeholders in understanding the complex environment associated with GES and the barriers and opportunities for its achievement, is exemplary for moving forward in MSFD implementation.

Cost-Effectiveness of Monitoring

Building on the ecological criteria for monitoring developed in Queirós et al. (2016), we developed an approach that uses multi-criteria-decision-analysis (MCDA) for cost-effectiveness analysis incorporating both ecological and economic criteria as attributes of monitoring systems. This approach encompassed a standardized scoring system for each of the different attributes, readily adaptable to the analysis undertaken with the attributes and the scores used as input to the MCDA. The cost-effectiveness of a given monitoring approach can be determined using the Rapfish software (www.rapfish.org), a non-parametric multivariate analysis tool, developed and tested in different contextual case studies of MSFD monitoring in Finland, Spain, and the UK. We also developed flow charts to help users identify the different elements of operational costs during monitoring. The tool can be applied to examine both the cost-effectiveness of the different monitoring elements and whether the monitoring programmes satisfy the requirements of the MSFD monitoring objectives. The tool has demonstrated, for example, a mixed ability of current monitoring programmes in Bay of Biscay to comply with the need to monitor changes in quality and quantity of different MSFD Descriptors. In addition, monitoring open sea areas in the Gulf of Finland becomes more cost-efficient when combining monitoring with research cruises on scientific vessels, which make up the largest single monitoring cost.

Cost-Benefit Analysis of New Management Measures to Achieve GES

By 2015, EU Member States had to define the Programme of Measures, including new measures if any, required to achieve GES. Oinonen et al. (2016a) stated that "the specific application of methods and uptake of resulting information are currently still evolving in the ecosystem-based and adaptive management framework that the Directive stipulates." They further recommend the use of environmental economics delivered through interdisciplinary research to support the needs of MSFD.

Three different case-studies showed interdisciplinary approaches to the cost-benefit analysis of management measures to achieve GES. In Finland, a quantitative cost-effectiveness analysis of implementing different management measures, based on opinion of interdisciplinary experts, identified the costs, and most cost-effective measures. Researchers estimated economic benefits of the management measures based on existing valuation studies (i.e., willingness to pay) on the benefits of improving the state of the Baltic Sea; these analyses connected the benefit estimates directly to the change in the status of the GES descriptors (Oinonen et al., 2016b). Extending from this analysis into a full cost-benefit analysis, the net value of achieving GES for indicators of biodiversity, food webs, and eutrophication alone in 2020 is placed at ∼2 bn € (although the planned management measures will not achieve GES of these Descriptors by 2020).

Alternative approaches to cost-benefit analysis of management measures were developed and applied in the Bay of Biscay and the East Coast of England Marine Plan Areas (ECE). These approaches built on research to determine changes in ecosystem services and the benefits that identified, mapped and modeled ecosystem services, and considered valuation of their benefits (e.g., Hattam et al., 2014, 2015; Galparsoro et al., 2014; Borja et al., 2015; Kleisner et al., 2015; Laurila-Pant et al., 2015).

The Bay of Biscay approach examined the links between ecosystem services and their benefits and management measures to control the development of maritime activities creating those benefits. We used the Fishrent bioeconomic model (Salz et al., 2011) to quantitatively assess the impacts, in terms of percentage changes in net present value, of implementing some of the measures under the European Common Fisheries Policy (CFP) expected to support attainment of GES.

The ECE approach used structured analysis of changes in ecosystem services and benefits arising from potential new management measures (ballast water treatment, underwater noise reduction) to identify the benefits of achieving GES alongside the costs of implementing the measures. Insufficient availability of valuation data, needed to quantify ecosystem benefit impacts in monetary terms, precluded the possibility of extending the analysis into a quantitative cost-benefit analysis.

Conclusions

Ensuring that Member States implement sufficient and non-overlapping measures to achieve GES will require the continuous review of legislation and policy, and the assessment of its implementation (Boyes et al., 2016). Furthermore, effective stakeholder engagement is likely to facilitate the acceptance of the measures and associated costs. The effective application of the MSFD requires knowledge and databases but these currently are limited by economics (Borja and Elliott, 2013). It remains to be seen how the different Member States identify the additional measures needed to improve the marine environment toward GES and close the gap between current status and GES in 2020. However, Member States must use existing budgets carefully to avoid further economic hardship resulting from financial penalties due to legal infraction proceedings in the European Court. For example, reduced funding for monitoring, if not guided toward more effective monitoring tools (see Section Monitoring Networks in European Regional Seas: Is Traditional Monitoring Sufficient to Assess the Status of Marine Ecosystems?), can reduce the quality of monitoring (e.g., by reducing spatial and/or temporal coverage). Such reduction can ultimately entail a greater cost than investment in monitoring as inaccurate evaluation could increase the risk of decision-making errors, potentially resulting in reduced ecosystem services and a devaluation of ecosystem benefits. Political decision makers may consider other aspects of monitoring as societally important, such as maintaining a bank of knowledge, technological development, professional skill and experience development and enhancing public engagement. Tools for determining the cost-effectiveness of monitoring and of management measures, as well as use of the ecosystem service approach to determine ecosystem benefits in cost-benefit analysis can support decisions on activities undertaken to comply with the MSFD. However, many member states implementing the MSFD lack both the data required to underpin rigorous economic analysis of costs of monitoring and the valuation data for assessing changes in ecosystem benefits from improvements in ecosystem services. Furthermore, the relationship between MSFD indicators and ecosystem services still requires better understanding and the implementation of the MSFD still urgently requires such data and information.

FILLING IN THE GAP BETWEEN SCIENCE AND POLICY

Some of the challenges in marine ecosystems ecology identified by Borja (2014) relate to socio-ecological topics, especially given the recognition of humans (and the activities they perform and pressures they pose in the oceans) as an integral part of the marine ecosystem in recent decades. The human dimension of marine systems remains poorly documented, and discussions

TABLE 2 | Progress beyond the state-of-the-art achieved by DEVOTES, within the different topics addressed by the project, and gaps bridged in science-policy.

Topic addressed	State in 2012	Progress in 2016, after DEVOTES	Gaps bridged in science-policy by DEVOTES	Challenges for the future
Understanding of human impacts at sea, including climate change	-DPSIR framework and derivatives - Scarce knowledge on climate change effects on MSFD	-DAPSI(W)R(M) approach Expansion of the framework to accommodate multiple activities, multiple pressures and mechanisms - Potential effects of climate change on the MSFD known - Matrices of pressure/impact for European regional seas	-Better understanding of the effects of human impacts at sea and the endogenic and exogenic pressures that can or cannot be managed	-Managing multiple pressures under climate change
Use of indicators in the assessment	-indicators identified to be needed for holistic ecosystem based approach for assessment of GES (European Commission, 2010) - No clear criteria for the selection of indicators - Lack of overview what indicators are currently available and well suitable for MSFD assessment	- DEVOTool available - >600 indicators collated and evaluated for their applicability for MSFD assessment - Gaps identified to further improve and develop indicators to cover needs of MSFD assessments - Criteria to develop and select ecologically relevant and robust indicators for MSFD assessments - 29 indicators developed or refined as a contribution to support concise indicator based assessment ecological status	- Availability of operational and scientifically sound criteria to select suitable indicators depending of the needs of the users - Availability of a suite of new and refined indicators to assess the status of marine waters, based on the gaps identified and to supplement the needs of the users - Publicly available user-friendly tool to select and rank indicators, depending on the operational needs of the marine managers in different regional seas, and available also for other stakeholders to evaluate GES assessments	- Develop environmental targets and reference conditions for some of the new indicators (and refine those of the old ones) to make them comparable across regional seas - Keep the DEVOTool database updated, and to provide support (help desk) for end-users of the tool
Modeling of marine systems	- Little use of models within the MSFD	- List of models suitable for the MSFD - Additive model for cumulative impacts of alien species - New developments in model ecosystem functioning - New approaches using mass-balanced models - Habitat modeling for different species - Models of connectivity developed	- Modeling studies support the use of indicators and functional linkages between ecosystem components and overwhelming pressures on the marine environment, such as climate change and ocean acidification, are more fully grasped and can be used in management - A series of case studies illustrating that modeling can assist the implementation of the MSFD, contributing in each step of the assessment and management cycle.	- Need of more integrative models at ecosystem level, covering all European seas - Need to better communicate to managers the usefulness and need of using modeling tools to implement the MSFD - Need to take uncertainty into consideration
Monitoring of marine systems	- Little knowledge regarding marine biodiversity monitoring networks on place at Pan-European scale - Traditional monitoring undertaken by EU Member States	- Catalog with information on marine biodiversity monitoring networks on place in the four European Regional Seas and their sub-regions - New molecular tools used to monitor and assess the status in microbes, plankton, meio- and macrofauna - Use of ARMS and ASUs to monitor hard-bottom - New applications of remote sensors to assess eutrophication - New biosensor as early warning of contamination	- Information on monitoring networks compiled and publically available - Identification of gaps and needs for further monitoring by European regional sea and marine sub-regions Traditional monitoring, regarded as too expensive to cover large areas, has been complemented with wide-scale and rapid surveillance techniques, useful for assessment and management	- Achieve coordinated monitoring within regional seas - Need to cooperate with non-EU countries, particularly in the Black and Mediterranean Seas - Need to optimize resources and apply the new monitoring tools under routine monitoring frameworks

(Continued)

TABLE 2 | Continued

Topic addressed	State in 2012	Progress in 2016, after DEVOTES	Gaps bridged in science-policy by DEVOTES	Challenges for the future
Assessment of marine systems	- Lack of an operational definition of GES - Little knowledge on aggregation methods - No integrative assessment tools available	- Proposal of an operative definition of GES - Proposal of different methods for aggregation - New integrative assessment tool (NEAT)	- Provision of the necessary basis to better understand what GES mean, when it is achieved and how can it be assessed, for a better management	- Integrate the assessment of ecosystem services in NEAT - Make assessments across regional seas comparable and harmonized
Economic and social dimensions	No knowledge of the barriers to implementing MSFD No tools to consider the cost-effectiveness of marine monitoring No approaches for undertaking cost-benefit analysis of management measures to improve the marine environment	Understanding of the initial governance, legislative and socio-economic barriers Systemic modeling approach to consult with stakeholders to overcome barriers Tool to undertake analysis of cost-effectiveness of monitoring Approaches to undertake cost-benefit analysis of management measures to achieve GES	Decision support on best use of limited resources for monitoring and for development of management measures Approaches to reveal the benefits of achieving GES enabling cost-benefit analysis of management measures that support decision making on approaches to achieve GES, potentially including motivation to do so.	Requirement for economic data on: - Costs of monitoring - Costs of implementing management measures - Valuation data to apply to benefits from ecosystem services - Bioeconomic modeling to support economic analysis of marine environmental benefits

DPSIR and DAPSI(W)R(M): D, drivers; A, activities; P, pressures; S, change of state; I, impact; W, human wellbeing; R, responses; M, management; MSFD, Marine Strategy Framework Directive. GES, Good Environmental Status; NEAT, Nested Environmental status Assessment Tool; ARMS, Autonomous Reef Monitoring Structures; ASU, Artificial Substrate Unit.

on ecosystem-based management of seas often minimize the importance of social sciences (Fréon et al., 2009), despite the explicit role of humans in implementing the Ecosystem Approach since its adoption in the Convention on Biological Diversity (CBD, 1992). Despite progress in recent years in connecting natural and social sciences, the gap between science (social and natural) and policy remains large in marine research (Nicholson et al., 2012). Europe has made efforts to close the research project-policy circuit in relation to the WFD (Oliver et al., 2005; Quevauviller et al., 2005; Hering et al., 2010), although until recently, few attempts have been made to close such a circuit for the MSFD (Borja et al., 2010).

Many challenges remain in bridging the gap between science and policy to support improved policy decisions in marine management (von Winterfeldt, 2013; Choi et al., 2015). As noted by Rodwell et al. (2014), improvement would require identifying: (i) the gap or perceived gap between marine science and policy; (ii) the obstacles that prevent us from bridging the gap, and (iii) the possible solutions.

More than 30 years ago, Sebek (1983) identified some of the reasons for marine public policy failure in incorporating scientific knowledge, but researchers since have removed some of the impediments (**Table 2**). Specific examples include:

(i) Lack of international regulation encompasses both EU and non-EU countries adjacent to the regional seas, which the different RSCs and, especially, the WFD and MSFD has overseen in recent years. DEVOTES brought together different pieces of legislation, identified gaps and overlaps and provided advice on future needs for the satisfactory implementation of the MSFD for the new Commission Decision expected in the coming months (Patrício et al., 2014);

(ii) Although monitoring increased after enacting the WFD and should continue to improve during the MSFD implementation (Patrício et al., 2014; Patrício et al., 2016b), it remains insufficient. DEVOTES contributed by identifying, testing and validating multiple innovative tools for monitoring and modeling large areas;

(iii) Independent scientific input into international conferences provided a means to organize multiple stakeholder meetings and offer training courses and sessions at international conferences aimed at disseminating and making project findings operational;

(iv) Incorporating scientific advice into regulations requires political compromise and we contributed by developing tools to assess environmental status (NEAT) that we hope will be incorporated into the implementation of the environmental quality directives (e.g., MSFD and WFD). Furthermore, a series of workshops and webinars with key stakeholders and end-users (e.g., policy-makes, relevant Member State representatives, RSC, etc.) has accompanied this development for all to understand the basis, capacities and functioning on this tool;

(v) Insufficient use of economic analysis to assess and support implementation of MSFD exacerbated a lack of appropriate monitoring costs, impacts and valuation data relevant to the assessment of marine ecosystems, their services and benefits. We developed new approaches to undertake cost effectiveness and cost- benefit analysis of monitoring and management measures, respectively, in the context of MSFD. Furthermore, we explored linkages between

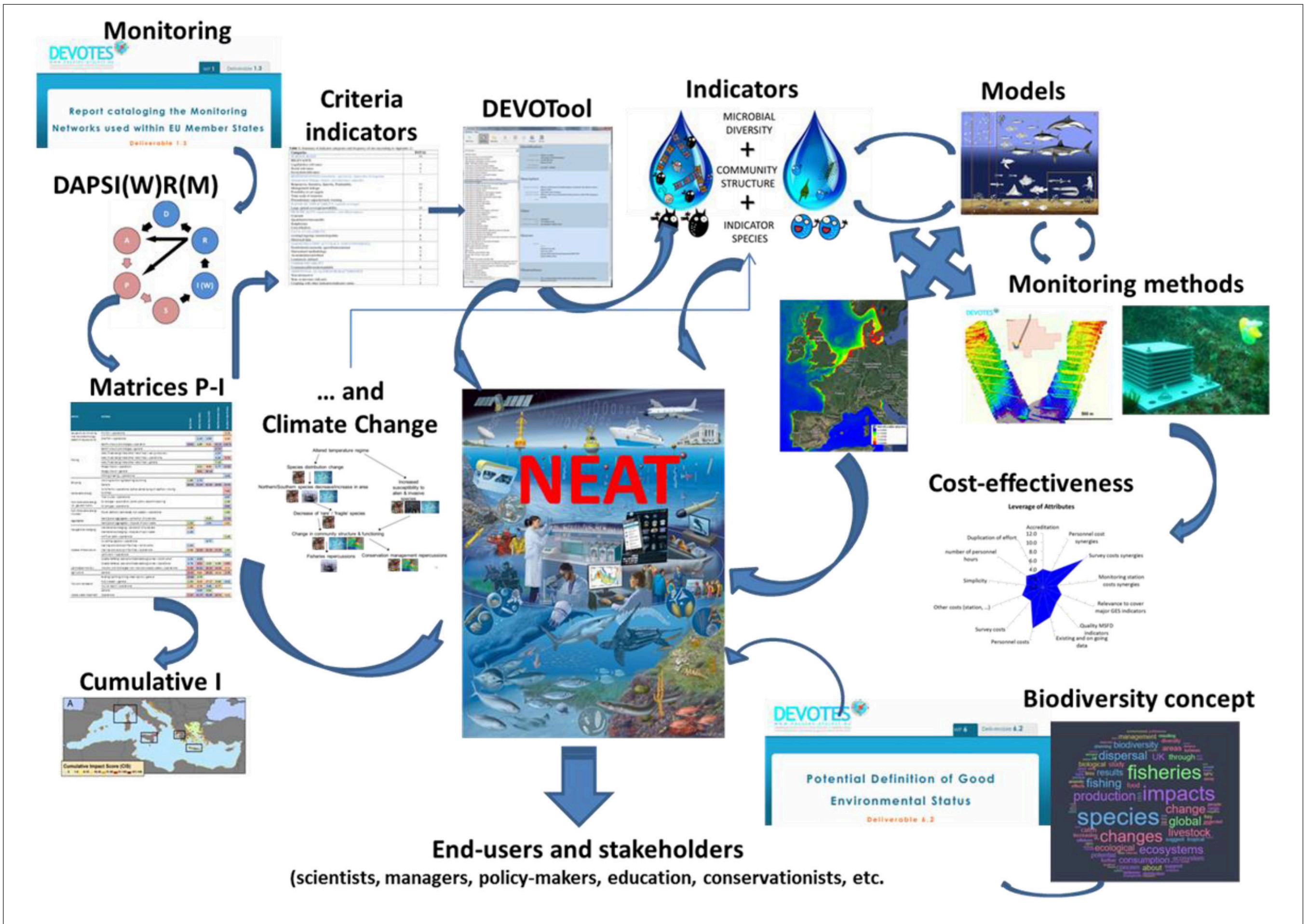

FIGURE 1 | Synthesis of the knowledge generated within the DEVOTES project, to assess the status of marine waters in an integrative manner. D, drivers; A, activities; P, pressures; S, change of state; I, impact; W, human wellbeing; R, responses; M, measures. NEAT, Nested Environmental status Assessment Tool. The central plate, about NEAT, was drawn by Alberto Gennari.

ecosystems and provision of ecosystem services as well as between management measures and ecosystem services.

DEVOTES was conceived as an integrative project that aimed to expand and merge natural and social sciences, enable the natural scientists to understand the economic and legal requirements and economic and governance specialists to understand the limitations of natural science. **Figure 1** encapsulates the work done and the integration of pieces to assess the status and respond to multiple stakeholders and end-users (i.e., scientists, policy-makers, managers, industry, conservation organizations, and society). Hence, our outputs not only increased knowledge of marine assessment and assisted marine managers, but also communicated these findings to increase stakeholder uptake.

In connection with the development of the MSFD programme of measures, Oinonen et al. (2016b) developed a pragmatic approach to holistic cost-effectiveness analysis. This allows users to select a cost-effective set of candidate measures in order to reach the multidimensional environmental objectives of the MSFD. They concluded that the major challenge in applying cost-effectiveness analysis was in assessing the current state of the environment and the multiple effects of different measures in evaluating marine ecosystem components rather than in the concepts of economic analysis. Despite this, economics helped to determine socially optimal level of marine protection.

Many challenges remain despite the body of work undertaken over the last 4 years (**Table 2**). These challenges include, among others: (i) understanding how multiple pressures act in marine ecosystems, and managing those pressures in the context of climate change; (ii) identifying key indicators and setting targets and reference conditions for those indicators so they can be used in assessments, noting the need for comparability across regional seas, and recognizing scenarios of climate change that shift baselines; (iii) the need to develop models capable of operating at an ecosystem level, with powerful computational capacities able to handle big data; (iv) getting EU Members States to consider adopting new monitoring tools routinely, and coordinating monitoring activities within regional sea research activities; (v) the need for intercomparable and harmonized assessments across regional seas that include ecosystem services in the assessment, and (vi) the urgent need for a harmonized framework under which indicator development follows specific rules and aligns with specific criteria to readily use in an integrative assessment tool.

GENERAL CONCLUSIONS

Defining, attaining and maintaining the GES of the seas spans from the technical details of monitoring and indicator implementation to major social and economic issues of how to optimize the long-term delivery of ecosystem goods and services, and how to govern society fairly in relation to use of the sea. It requires integrating natural and social sciences, horizontal integration across stakeholders, and vertical integration through governance, and feedback between monitoring, measures, and management. The MSFD aspires to bring all these aspects under the same umbrella, an ambitious and highly relevant objective.

DEVOTES advanced the state-of-the-art and identified major gaps within various aspects of MSFD implementation, contributed to filling these gaps, and identified additional scientific and development needs. The further development and validation of marine biodiversity indicators requires improved data with better spatial and temporal coverage, based on novel and cost-efficient monitoring methods. Better ecological relevance and indicator responsiveness to pressures will require experimental research on different levels of biological organization from the cell to the ecosystem. Such research will also enable incorporation of indicators into models in order to extrapolate marine assessment results to larger spatial and temporal scales. Each of these aspects requires comprehensive and integrated natural and social sciences which cross international boundaries and regional seas.

AUTHOR CONTRIBUTIONS

AB conceived the paper and wrote the first draft; ME, AB contributed to the pressures section; TB, SC, MM, AH, JP, LU contributed to the indicators section; CL, CW contributed to the modeling section; RD, AB, AN, JP, SC, PS, MU contributed to the monitoring section; JC, AB, TB, SC, MU contributed to the integration section; MA, MU contributed to the socio-economic area; all authors contributed equally to the discussion and conclusions, AB, ME, SG, PS, MU made a revision of the whole text.

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