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A systematic study of zooplankton-based indices of marine ecological change and water quality: Application to the European marine strategy framework Directive (MSFD)

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ABSTRACT

Marine zooplankton are central components of holistic ecosystem assessments due to their intermediary role in the food chain, linking the base of the food chain with higher trophic levels. As a result, these organisms incorporate the inherent properties and changes occurring atall levels of the marine ecosystem, temporally integrating signatures of physical and chemical conditions. For this reason, zooplankton-based biometrics are widely accepted as useful tools for assessing and monitoring the ecological health and integrity of aquatic systems. The European Marine Strategy Framework Directive (EU-MSFD) requires the use of different types of biomonitors, including zooplankton, to monitor progress towards achieving specific environmental and water quality targets in EU. However, there is currently no comprehensive synthesis of zooplankton indices development, use, and associated challenges. We addressed this issue with a two-step approach. First, we formulated the indicator-metrics-indices cycle (IMIC) to redefine the closely related but often ambiguously utilized terms - indicator, metric and index, highlighting the convergence between them and the iterative nature of their interaction. Secondly, we formulated frameworks for synthesizing, presenting and systematically applying zooplankton indices based on the IMIC framework. The main benefits of the IMIC are twofold: 1). to disambiguate the key elements: indicators, metrics, and indices, revealing their links to an operational ecological indicator system, and 2) to serve as an organizing tool for the coherent classification of indices according to the MSFD descriptors. Using the IMIC framework, we identified and described two broad categories of indices namely the core biodiversity indices already in use in the Baltic Sea and North Atlantic regions, including the 'Zooplankton Mean Size and Total Stock (zooplankton MSTS)' and 'Plankton Lifeforms index (PLI)', and stressor-response indices retrieved from the existing literature, elucidating their applicability to different MSFD descriptors. Finally, major challenges of developing new indices and applying existing ones in the context of the MSFD were critically addressed and some solutions were proposed.

1. Introduction

Marine ecosystems face increasing pressure from multiple simultaneous stressors (Eggermont and Martens, 2011). The fast-changing abiotic environment coupled with mounting pressure from human-environment interactions and accelerating climatic change render efforts to capture and quantify multiple stressor impacts on marine environments using only traditional physicochemical approaches increasingly difficult (Holt and Miller, 2010). These challenges necessitate the development of methods enabling the systematic

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identification of the underlying drivers and stressors of ecosystem alterations in time and space. One of such methods popularized over the past five decades is the use of ecological indicators to detect ecosystem changes and monitor progress towards environmental policy targets (Smeets and Weterings, 1999; Niemi and McDonald, 2004; Holt and Miller, 2010; Zampoukas et al., 2014). The Marine Strategy Framework Directive was promulgated to support the coherent and harmonious implementation of European Commission Directive 2008/56/EC (MSFD Common Implementation Strategy, 2017), via intensified cooperative efforts aimed at achieving or maintaining a 'good environmental status' (GES) using a set of environmental and ecological indicators/criteria implemented via six-year management cycles (European Commission, 2008; Zampoukas et al., 2014; Walmsley et al., 2016; European Commission, 2017; Commission, 2020a; MSFD Common Implementation Strategy, 2017; European Commission, 2018; Borja et al., 2021). Article 3 of the Directive defines marine waters in a state of GES as those that are clean, healthy, productive, ecologically diverse and dynamic, where multiple simultaneous uses are conducted at a sustainable level, ensuring their continuity for future generations (European Commission, 2008, 2010, 2017, 2018; Commission, 2020a). Initially, the MSFD Decision 2010/477/EU laid down a set of 56 indicators and 29 criteria linked to 11 qualitative descriptors to help Member States implement environmental status assessments and monitor the extent to which GES was achieved in their marine waters. The subsequent European Commission Decision (EU) 2017/848 adopted on May 17, 2017, replaced the 2010 legislation, introducing new criteria and methodological standards on GES determination as well as specifications, methods and technical guidelines related to monitoring and assessment (Walmsley et al., 2016; European Commission, 2017; MSFD Common Implementation Strategy, 2017). To implement the various criteria and meet the MSFD goals , planktonic indicators have gained traction in recent decades as the EU Member States are now required to include phytoplankton and zooplankton to their marine environmental monitoring and assessment programs (Gorokhova et al., 2016) to aid in the diagnosis of ecosystem disturbances and determine ecosystem integrity. The reason for the increasing use of planktonic organisms in marine ecological assessments is that these organisms reflect and integrate ecological and environmental conditions by their quick and perceptible responses to changing abiotic parameters (Thackeray et al., 2016; Batten et al., 2019). This interest in biological monitoring as an ecosystem assessment tool has stimulated the development of a number of biotic indices including plankton-related ones (HELCOM, 2006, 2010; HELCOM, 2013a; Pawlak et al., 2009), alongside abiotic parameters (HELCOM, 2006; Tett et al., 2007). Phytoplankton, in particular, have been widely used as an indicator of nutrient status in contemporary aquatic ecosystems (Tett et al., 2007; Tett et al., 2008; Garmendia et al., 2013), as well as to infer past environmental conditions in paleo-research (Hou et al., 2014; Carballeira and Pontevedra-Pombal, 2020; Wang et al., 2021). For instance, blooms of *Phaeocystis pouchetii* in the North Sea (Johns and Reid, 2001) and the ratio of diatoms to dinoflagellates in the Baltic Sea (Klais et al., 2011; HELCOM, 2013a; OSPAR, 2017b), were used to characterize the eutrophication status of these environments. Fish and macro-invertebrates have also received significant attention asbio-indicators (Harrison and Whitfield, 2004). In contrast, zooplankton have rarely been included in biomonitoring schemes and thus have historically lagged the other bio-indicators. . However, in recent years, the systematic development, coordination and use of zooplankton indicators have increased substantially (Caroppo et al., 2013; Wasmund et al., 2016; ICES, 2018), due largely to the requirements of the MSFD to include plankton to the descriptors of GES especially those related to biodiversity, food webs and eutrophication (Gorokhova et al., 2013; Gorokhova et al., 2016; HELCOM, 2015; McQuatters-Gollop et al., 2019). The most compelling scientific reasons for using zooplankton as bio-monitors is their intermediary role in the aquatic food chain linking primary producers with higher trophic levels, driving biogeochemical cycling (Zannatul and Muktadir, 2009), contributing to energy transfer in pelagic food webs, and ultimately affecting fish recruitment and other ecosystem services (Margoński, 2007; Caroppo et al., 2013; HELCOM, 2015; McQuatters-Gollop et al., 2019). At the same time, zooplankton are sensitive to environmental conditions and respond perceptibly to changes in water temperature, chemistry and other hydrographic factors (Šorf et al., 2015; Rasconi et al., 2015). Despite, the demonstrated value of zooplankton as bio-indicators of water quality and eutrophication status (e.g. Webber and Webber, 1998; Buchanan, 1993; Olson et al., 2005), the wide-scale development and application of such indicators is still relatively recent and faces numerous challenges. A common and more general challenge in the context of the MSFD is the ambiguous use of the key terms - indicators, indices and metrics, affecting our ability to understand, develop and effectively apply indices to the various MSFD GES descriptors. Furthermore, pressure indicators that describe ecological status based on the response of particular zooplankton species to stressors have not been effectively addressed by regional efforts to implement the MSFD. This category of indicators is vital for understanding the current state of marine ecosystems and for predicting future ecological changes, but the available information is currently uncoordinated and scattered across the literature. In the present study, we began by redefining and organizing the triple concepts: indicators, metrics, and indices to address the ambiguity associated with their use in the literature. We then reviewed the existing zooplankton indices, highlighting their indicator value and applicability to the MSFD. We recommend this structural approach to support future work on developing and applying zooplankton indices and especially to facilitate the implementation of the MSFD.

2. Methodology

We employed a systematic qualitative approach through narrative analysis and synthesis to describe the current knowledge on the development and application of zooplankton indicators and indices in environmental assessments and monitoring. We conducted comprehensive searches of multiple peer-reviewed sources and non-peer-reviewed databases related to the MSFD. The non-peer-reviewed sources included web information, progress reports and student dissertations (e.g., Bolte, 2013; HELCOM, 2015, 2018; Holgate, 2016; OSPAR, 2017a; OSPAR, 2017b) and catalogues (e.g. DEVOTES Project, 2014; AquaNIS. Editorial Board, 2015). The primary search tools were Google scholar and an intuitive graph-based tool known as 'connected papers' (https://www.connectedpapers.com/). The latter tool allowed us to quickly identify the most pertinent articles, access their titles and abstracts, and screen these to determine their relevance to this study's overall objectives. The search for articles was conducted using multiple search terms including indicator, metrics, indices, MSFD, zooplankton indicators of nutrient enrichment, zooplankton indicators of hydrographic changes, zooplankton indicators of climate change, and zooplankton indicators of pollution. Our literature search resulted in 350 references. Out of those, we identified 190 relevant articles, including 30 non-peer-reviewed contributions. We synthesized and summarised the data in a way that illustrates the effective use of the terms indicators, metrics and indices, and categorized available indices into biodiversity indices and the pressure-response indices found across the extant literature.

3. Revisiting the core concepts: The Indicators-Metrics-Indices Cycle (IMIC)

The MSFD has defined a wide range of descriptors that must be operationalized by member states of the EU to implement monitoring and assessment procedures of their specific marine regions (German Federal Agency for Nature Conservation and the Federal Environment Agency, 2011). A major challenge in this regard is that the concepts: indicators, indices and metrics, are often used interchangeably in the literature, creating ambiguity that limits their understanding and application. For instance, the ambiguous use of the term indicators in

European Commission Decision 2010/477/EU and related publications e.g., the DEVOTool catalogue [Annex 1]), resulted in incoherencies and misunderstandings on the determination of GES across the EU Member States. Consequently, Decision 2010/477 was replaced with the European Commission Decision (EU) 2017/848 that substituted indicators with quantifiable primary and secondary criteria and elements linked to each of the 11 GES descriptors and developed methodological guidelines for assessing whether GES has been achieved (Walmsley et al., 2016; European Commission, 2017).

We noticed that the key terms metrics and indices are still grouped under the umbrella term 'indicators'. We argue that it is necessary to further disambiguate and redefine the main concepts to facilitate the MSFD implementation process. For this purpose, we developed an iterative framework the 'Indicator-Metric-Indices Cycle (IMIC)' that highlights the distinctions between these terms while illustrating their interlinkages in the context of the MSFD. We concur with the definition of an indicator as a proxy intended to highlight the general ecosystem status and communicate information on specific phenomena or complex processes in a simplified and aggregated manner (Pletterbauer et al., 2016; Chiba et al., 2018). This definition corresponds to MSFD Article 9 (1) of Decision 2017/848 defining indicators as scientifically-based expressions that provide an operational dimension to GES criteria as a means to assess the extent to which GES has been achieved (European Commission, 2020b). Thus, indicators represent simplified abstractions of each MSFD environmental status descriptor allowing for subsequent change in ecological attributes to be monitored over time (Zampoukas et al., 2014). Here, we posit that indicators in themselves are not robust quantitative parameters because of the amount of diverse information they contain, but the specific measurable traits derived from them such as abundance, biomass, weight and size, are the quantitative dimensions are metrics, defined as numerical expressions of indicators that quantify aspects of the population structure, function or other measurable characteristics that change predictably under the influence of different types of pressures (Pykh et al., 1997; Zampoukas et al., 2013; Zampoukas et al., 2014; Pletterbauer et al., 2016). The quantitative data derived from metrics enable the identification of patterns and trends and the establishment of baseline conditions, subsequently used to develop mathematically robust indices that enable the state of a monitored system to be assessed and described with simple designations such as 'improved or improving', 'bad, better or worse' (Teixeira et al., 2014; Teixeira et al., 2016), representing a system's status relative to a pre-established reference condition.

The interaction of indicators, metrics and indices form the basis for an 'operational ecological indicator system'. The IMIC framework, therefore, dissociates the quantifiable metrics or criteria needed to develop useful indices from broadly defined qualitative indicators. Using zooplankton as indicator organisms, the IMIC represents a continuum of information from an abstract description of a system's state (indicators) to relatively more focused biometrics such as abundance, biomass, biovolume and other traits derived from specific zooplankton taxa (metrics) and finally to more specific indices often represented by a single value or range of values describing targeted components the ecosystem's condition. We used the different components of the MSFD to exemplify the IMIC consisting of four parts (Fig. 1). The first part comprises of the 11 qualitative descriptors (biodiversity – D1, alien species – D2, fish stocks – D3, food-webs – D4, eutrophication – D5, sea-bed integrity – D6, Hydrographical Changes – D7, contaminants in the sea – D8, contaminants in seafood - D9, litter – D10, and energy – D11) (Cardoso et al., 2010; Zampoukas et al., 2013; Zampoukas et al., 2014). Descriptors D1, D3, D4 and D5 constitute the 'state indicators' while the rests are 'pressure indicators' (Cardoso et al., 2010; German Federal Agency for Nature Conservation and the Federal Environment Agency, 2011). According to the IMIC, these are the first-tier indicators representing the holistic qualitative descriptions of the marine environmental conditions for which GES must be assessed or achieved. The second component of the IMIC represents quantifiable criteria that are

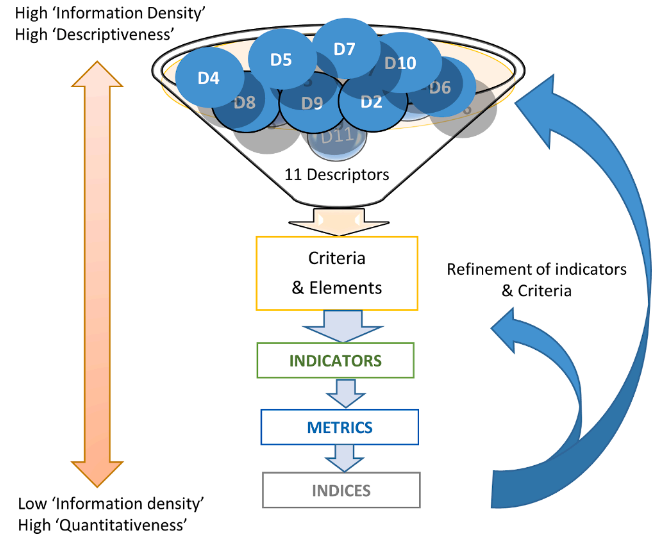

Fig. 1. A schematic of the MSFD hierarchical structure to exemplify the IMIC framework aimed at facilitating the assessing of marine environmental status. The first two levels depict the 11 qualitative descriptors of GES (D1-11) and the quantitative criteria as defined by the MSFD. The latter three levels highlight the operational component of the IMIC via indicators, metrics and indices.

deductions of the 11 qualitative descriptors (second-tier indicators). The first-tier (descriptors) are characterized by the highest level of 'abstraction, and 'information density', while the second-tier criteria provide more detail and direction to the GES assessment and monitoring parameters. The third tier of the IMIC comprises indicators. In the context of the present study, diverse zooplankton species constitute the indicators used to add a measurable dimension to the criteria. Metrics constitute the fourth tier of the IMIC and comprise specific zooplankton demographics (density, biomass, biovolume) and functional traits (individual size, age, sex) required to add a more robust quantifiable dimension to each previously defined or existing criteria and indicator. Finally, indices comprising the fifth tier of the IMIC are robust mathematical representations of different GES criteria based on the data derived from metrics (Fig. 1).

The newly developed indices should be used to assess the ecological status of the marine environment relative to each MSFD descriptor. The main benefit of the IMIC beyond for classification purposes is that it helps to disambiguate the key terms indicators, metrics, and indices, highlighting a continuum that is the basis for an operational ecological indicator system. Therefore, the framework is an organizing tool that ensures coherence in the entire process, from framing broad ecological questions and developing qualitative descriptors, identifying quantifiable metrics relevant to each descriptor, to the quantification of ecological status via robust indices.

The totality of the components of IMIC is required to operationalize the Ecological Indicator System. The first tier qualitative indicators of the MSFD fulfil the multi-purpose (i.e., capable of informing on more parameters, species, habitats or pressures) and the comparability (capable of being compared across neighbouring states considering regional differences) properties of a 'good ecological indicator system' while metrics and indices fulfil the rest of the properties of a good ecological indicator system namely i). Predictability of responses to anthropogenic pressures; ii). Statistical robustness, with a quantitative threshold value indicating GES/sub-GES, and iii). Cost-efficiency, per Zampoukas et al. (2014). The iterative nature of the IMIC (blue arrow, Fig. 1) elucidates the imperative to revise the second-tier indicators or update the existing monitoring strategy as new information concerning the ecological condition becomes available, analogous to the MSFD implementation cycle.

4. The typology of zooplankton indicators, metrics and indices

The MSFD requires EU member states to develop environmental targets and associated indicators to achieve GES in coordination with other member states that share a common marine region or sub-region. Two broad categories of criteria were defined to guide the GES assessment and monitoring processes among the EU Member States in association with the MSFD Descriptors, including the 'state' and 'pressure' criteria per Article 8(1 a, b) (Walmsley et al., 2016; MSFD Common Implementation Strategy, 2017). These criteria also serve to inform the setting of environmental targets, referring to the desired conditions or changes that are necessary to attain GES, including state-based targets, pressure-based targets, impact-based targets and operational targets, referring to the type of management actions needed to reduce the impact of pressures on marine environments and foster their recovery towards GES (Walmsley et al., 2016). In this context, zooplankton-based metrics and indices are recognized as a viable and coherent option for monitoring and assessing the MSFD State and Pressure descriptors of GES, ranging from holistic indices that integrate a broad spectrum of environmental information and reflect the system-wide status of ecosystem health and integrity, to more reductionist stressor-related indices (Caroppo et al., 2013), describing zooplankton communities' responses to specific stressors. In the following sections, we will first describe the core zooplankton state indices developed in the context of the MSFD, and then present several zooplankton-based pressure indices retrieved from the published literature, linking these to specific MSFD descriptors (first-tier indicators).

4.1. State indicators

The MSFD is implemented through regional seas conventions notably the OSPAR (Northeast Atlantic) and HELCOM (Baltic) conventions. The HELCOM CORESET project 2010–2013 proposed 20 core indicators for biodiversity and 13 for hazardous substances and their biological effects (HELCOM, 2013b). Among these, three zooplankton indices currently comprise part of the core indicators of HELCOM (HELCOM, 2013b) and the OSPAR intermediate assessment (OSPAR, 2017a; OSPAR, 2017b). These include the 'Zooplankton Mean Size and Total Stock (zooplankton MSTS)', the 'Plankton Lifeforms Index (PLI)', and metrics based on the arrival of new non-indigenous species (NIS), associated with the MSFD state Descriptors (D1, D3, D4 and D6).

4.1.1. The zooplankton mean size and total stock and related indices

The zooplankton mean size and total stock index (MSTS), HELCOM's core multi-dimensional index for characterizing the pelagic food-web structure and trophic efficiency (Margoński et al., 2007; Gorokhova et al., 2013; Gorokhova et al., 2016; HELCOM, 2018; Labuce et al., 2020) is based on the premise that mesozooplankton body sizes and biomasses are directly proportional to their grazing efficiency and trophic energy transfer efficacy (Simm et al., 2014; Holgate, 2016; Yebra et al., 2017). It evaluates whether good status is achieved using two threshold values for both metrics: mean size, and total abundance of zooplankton. The abundance or biomass of zooplankton relative to the reference condition can indicate marine productivity status (Buchanan, 1993), while size structure distribution indicates ecosystem structural changes. Related indices based on biomass metrics include the Mean Weight and Total Biomass Index (MWTB) (Simm et al., 2014), the Normalized Biomass Size-Spectra (NBSS) and the Abundance-Size Spectrum of zooplankton, referring to the relative abundance or biomass of zooplankton organisms of different size classes (Quintana et al., 2002; Thompson et al., 2013). The calculation of the zooplankton MSTS requires robust mathematical procedures applied to the metrics mean size, mean weight or total stock (biomass) of key zooplankton species, over time relative to the reference values derived for each of the metrics. Scatter plots containing two axes (mean size, Y-axis and total biomass, X-axis) are then derived to evaluate the environmental status and GES is seen to be achieved when the mean size and total biomass of dominant zooplankton communities do not deviate significantly from the predetermined reference values (Simm et al., 2014; HELCOM, 2015, 2018).

The zooplankton MSTS index and related indices have been applied to assess the strength of trophic linkages, detect trophic shifts and interactions, assess the success rate of fish larval survival and recruitment (Buchanan, 1993; Thompson et al., 2013), predict fish-feeding conditions, fish growth and size (Castonguay et al., 2008), and can indicate fundamental structural changes in the pelagic ecosystem linked to eutrophication (Gorokhova et al., 2013) (Fig. 2).

Pitois et al. (2021) applied the zooplankton MSTS concept to develop and test a 'Copepod Mean Size and Total Abundance index (CMSTA)' in the Celtic Sea. The authors explored the relationships between Copepoda mean size, total abundance and biomass with hydrographic and biological variables including Chlorophyll-a concentration and the biomass distribution of planktivorous fish. They found strong correlations between herring distribution and larger copepod mean sizes rather than high copepod abundances. Their results confirmed that copepods mean size has the potential to reflect food web and ecosystem health status as well as highlight climatic impacts on marine ecosystems. Similarly, the total 'biomass of large to small copepods was suggested as a reliable indicator of trophic status and productivity alterations in aquatic systems (Ramachandra et al., 2006). It is important to note that the purported ability of the zooplankton MSTS to infer specific ecosystem health stressors is predicated on the basis that the increase in zooplankton abundance coupled with a decrease in the mean size of zooplankton individuals are indicative of eutrophication (HELCOM, 2018). This might not always be the case, as size-based feeding preferences of higher trophic levels (fish and other predators) and a range of other environmental pressures (Simm et al., 2014), can also cause an ecosystem to become dominated by smaller-bodied individuals. A clear example that violates the above premise is the recent situation in the German Bight. In this ecosystem, the increase in larger-bodied copepods from the late 1970s to the early 1980s (Boersma et al., 2015) coincided with relatively high values of nutrients (N, P) higher than the background levels for the region (Brockmann et al., 2003; Brockmann et al., 2014). This was followed by a decline in the abundances of all copepods during the subsequent decades plus a decrease in average size (Boersma et al., 2015), concomitant with the significant decline in nutrient concentrations as a result of effective nutrient management measures (Brockmann et al., 2014). Therefore, although the practical assessment of biodiversity, food webs and trophic status can be achieved with holistic indices, the MSTS is not robust enough to establish the explicit links between the changing ecosystem structure and eutrophication or other specific ecological stressors. Hence, a stringent diagnosis of specific ecosystems is required before applying this index for marine ecological health assessments.

4.1.2. Plankton lifeform index

The plankton lifeform index (PLI) is another widely used core index developed for the Northeast Atlantic (OSPAR) region as a state indicator based on the premise that key planktonic lifeforms (functionally related species or taxa that are not necessarily taxonomically related) are veritable building blocks of biomes and can reveal broad structural and functional changes in marine ecosystems (Tett, 2016; Tett et al., 2003; McQuatters-Gollop et al., 2019). PLIs have also been used in the Baltic (HELCOM) and Mediterranean regions (Tett et al., 2008; Garmendia et al., 2013; Caroppo et al., 2013; Gorokhova et al., 2016; McQuatters-Gollop et al., 2019), and the Chesapeake Bay (Buchanan, 1993; Olson et al., 2005; Chesapeake Bay Consortium, 2005) to track top-down/bottom-up processes linked to shifts in the marine ecological structure. The Plankton Index (PI) is a robust computational tool used to calculate the PLI using 2-dimensional graphs to determine the condition of two plankton lifeforms Euclidian state-space relative to a reference period represented by a reference envelope drawn around a group of reference

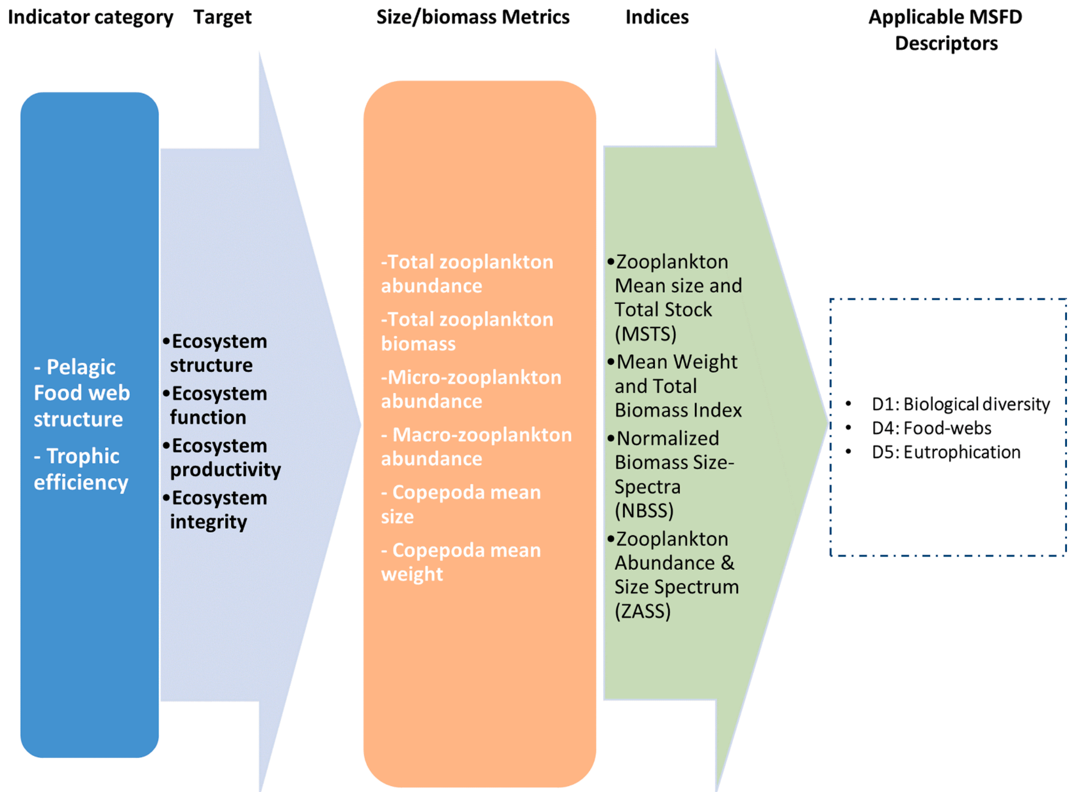

Fig. 2. A schematic of the zooplankton MSTS index and related holistic zooplankton-based metrics and indices.

points (Tett, 2016). Points falling inside or outside the envelope determine the condition of the pelagic ecosystem and possibly reveal the effects of eutrophication as a secondary link (Tett, 2016; Tett et al., 2003). However, the PLI was developed and applied primarily to detect broad ecological changes with no direct links to specific pressures. The PLI is therefore an overarching concept that includes phytoplankton and zooplankton lifeforms, e.g. the small and large copepods or the holo- and mero-plankton lifeform pairs, which can be assessed both individually and jointly. Demographic traits derived from relevant lifeform pairs (e.g. abundance, biomass and the size structure of zooplankton)

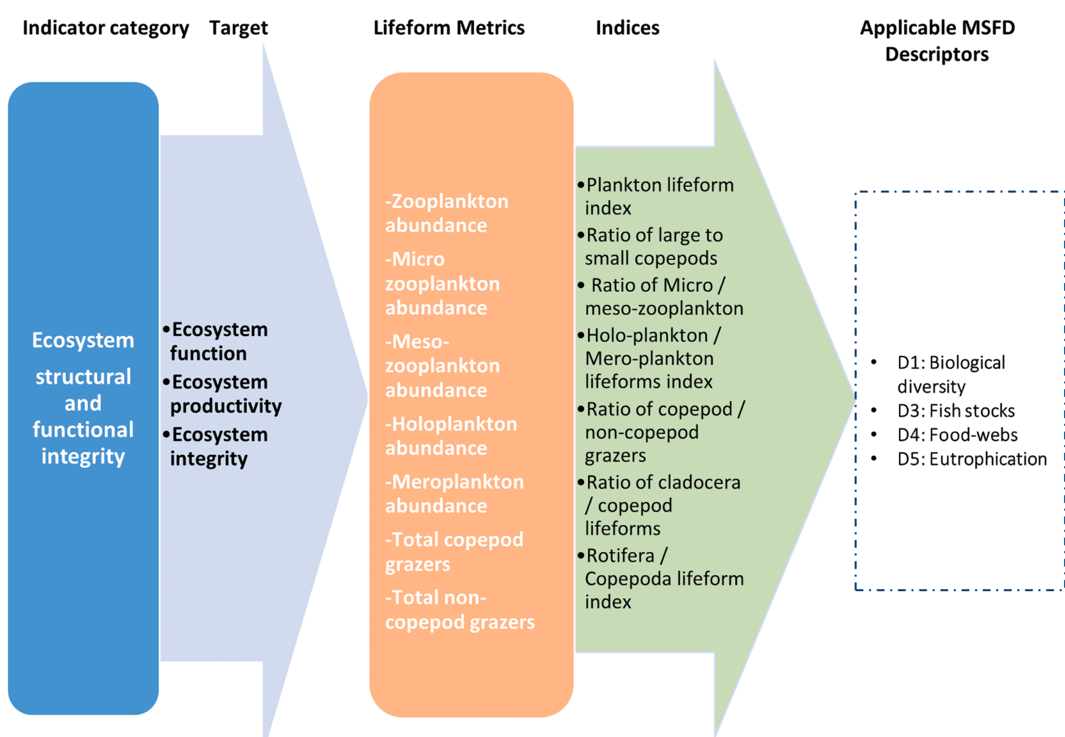

Fig. 3. Zooplankton lifeform indicators, metrics and indices of marine ecosystem functioning and productivity.

(Raghukumar and Anil, 2003; Jernberg et al., 2017) are also relevant metrics used to develop the PLIs for assessing pelagic food webs functioning (Fig. 3).

Assessments of zooplankton lifeform pairs can therefore reveal alterations in food-web structure and energy flows, and changes in mass and energy transfers between the benthic and pelagic components of the ecosystem, respectively (Margoński et al., 2007; ICES, 2016; Gorokhova et al., 2016; OSPAR, 2017b; Druon et al., 2019; McQuatters-Gollop et al., 2019). Similar to the zooplankton MSTS, the PLI has a secondary link to eutrophication (D5) because specific planktonic lifeforms or some functional traits can be highly sensitive and respond perceptibly to anthropogenic nutrient alterations (Tett et al., 2003). Overall, zooplankton holistic indices are highly relevant to the characterization of general ecosystem status (European Commission, 2010) and apply directly to the MSFD Descriptors D1 (Biological diversity), D4 (Food webs), and indirectly to D3 (Commercial fisheries), and can as well be relevant for assessing the status of the food web structure in response to eutrophication (D5) (Cardoso et al., 2010; HELCOM, 2015, 2018). However, due to their generalist nature, these indices do not possess sufficient indicator power regarding the impacts of specific ecological stressors linked to anthropogenic alterations and climatic change. This necessitates additional indices that specifically address stressor-response relationships in marine ecosystems. The following section describes some of these metrics and indices applicable to the MSFD to infer the ecological effects of multiple stressors in the marine environment.

4.2. Pressure indicators

According to the MSFD, marine environmental status assessments must reflect a range of pressures and their impacts on species/species groups, habitats, and can directly inform on the ecological status of a given assessment area, thereby further strengthening holistic state-based assessments. The importance of the pressure-related assessments is demonstrated by the fact that MSFD pressure descriptors (D2, D3, D5, D7, D8, D9, D10 and D11) outnumber the state descriptors (Walmsley et al., 2016). Hence, according to the 'background document on the determination of good environmental status', "MSFD implementation will be most effective when focused on the anthropogenic pressures that are preventing the achievement of GES, as management actions aimed at reducing the effects of these pressures will ultimately allow the marine environment to recover towards GES" (MSFD Common Implementation Strategy, 2017). Zooplankton-based pressure indices are therefore useful in targeting specific ecological issues and can infer physicochemical alterations that significantly impact marine ecological health. These include changes directly linked to large-scale human pressures such as nutrient enrichment, pollution and contamination, hydrographic alterations associated with multiple uses of the marine environment and low-frequency ocean-climatic variability and ocean acidification. These issues cover a wide range of MSFD Descriptors (D5-D11) and associated indicators. However, pressure indices have not been extensively developed to quantify the seven GES qualitative pressure indicators of the MSFD (Descriptors D2, D5, D7 and D8, D9, D10, D11) (Cardoso et al., 2010), compared to the state indices. Hence, we present here a synthesis of relevant pressure indices retrieved from the literature and relate them to various MSFD Descriptors (especially D2, D5, D7 and D8).

4.2.1. Indicators of ecological health

Marine pollution and contamination are among the dominant stressors of marine ecosystems linked directly or indirectly to human use or misuse of the environment. Environmental contaminants have far-reaching effects on different ecosystem levels, affecting biological processes, potentially altering taxonomic communities via direct cause and effect relationships (Law et al., 2010; Cardoso et al., 2010). However, although eutrophication and pollution are usually lumped together in a single category as water quality stressors, they are separate issues with different causes and ecological impacts, requiring different sets of indicators and metrics. The MSFD recognized this need by distinguishing both stressors into two descriptors, D5 (Eutrophication) and D8 (contaminants in the sea) (Fig. 4). According to D5, human-induced eutrophication and its adverse effects on biodiversity, including ecosystem degradation, harmful algae blooms and oxygen deficiency in bottom waters must be substantially minimized in European waters (Ferreira et al., 2011; Ferreira et al., 2010; Walmsley et al., 2016; MSFD Common Implementation Strategy, 2017). However, at present, chemical abiotic parameters and phytoplankton/macrofauna metrics are the main assessment parameters proposed to address D5 and its related criteria C1-C8. These parameters relate to the pressure (nutrient concentration in the marine environment – D5C1) and effects (D5C2–8 address the effects of eutrophication in the water column and on the seabed) (Walmsley et al., 2016). The MSFD has not explicitly linked zooplankton metrics to eutrophication and water contamination (Law et al., 2010). Furthermore, although zooplankton-based HELCOM/OSPAR core indicators - zooplankton MSTS and the PLI - are often suggested as also capable of indicating eutrophication on the premise that the dominance of small-bodied copepods is characteristic of eutrophic conditions, these are only secondary eutrophication indicators due to their low stressor specificity. Nonetheless, there is sufficient evidence in the literature attesting to the potential of zooplankton in eutrophication assessment. Therefore, to identify potentially reliable zooplankton-based bio-indicators of eutrophication and contamination, it was necessary to rely on studies that have specifically linked certain zooplankton species or communities to specific water quality changes linked to chemical and other pollutants.

4.2.1.1. Zooplankton-based indices of eutrophication. Many marine zooplankton species and assemblages are demonstrably sensitive to different forms of water quality changes, making them suitable candidates as eutrophication indicators. Experimental evidence in freshwater and nearshore coastal environments reveals that nutrient enrichment (Ejsmont-Karabin, 2012; Krupa et al., 2020), changes in water chemical properties (e.g. pH and alkalinity) (Tessier and Horwitz, 1990) and reductions in salinity modify zooplankton community structure towards the dominance of rotifers, cladocerans and small copepods. The Rotifer-Trophic State Index (RTSI) is particularly popular as a zooplankton index of ecological health based on the notion that rotifers are sensitive to and increase rapidly under eutrophic conditions (Buchanan, 1993; Steinberg and Saba, 2008; Ejsmont-Karabin, 2012; Gutkowska et al., 2013). This index combines Carlson's trophic indices and rotifer-based metrics (e.g. the abundance and biomass) analysed using regression methods to evaluate water quality changes (Gorokhova et al., 2013). Other existing trophic state indices are based on the ratios of pairs of ecologically relevant zooplankton communities including the *Rotifera: Copepoda* ratio (Olson et al., 2005), the *Cyclopoida: Calanoida* ratio and the Crustacean Trophic State Index (CTSI) (Ejsmont-Karabin and Karabin, 2013) used in different regions to indicate changes in eutrophication status (Ejsmont-Karabin, 2012; Suliman et al., 2017). The shift in planktonic copepod's size-spectra and diversity along the offshore - nearshore gradient was also perceived as an indicator of deteriorating water quality linked to high phosphorus levels, turbidity and increased chemical oxygen demand (eutrophication) (Drira et al., 2018). We however realize that indices based on *Rotifera* and *Cladocera* are almost exclusively applied to fresh- or brackish water ecosystems (e.g. Parmar et al., 2016; Jurczak et al., 2019; Diwen et al., 2020). Our decision to highlight them here is based strictly on their potential to become vital diagnostic tools for future marine ecosystems under the influence of climatic and anthropogenic changes and potential marine freshening. Furthermore, Rota et al. (2009) found that the decline of the cephalochordate *Branchiostoma lanceolatum* along the Italian coasts indicated changes in environmental quality likely linked to chemical pollution and eutrophication. Salinity decline and the increase in muddy sediments have also been advanced as important stressors limiting the distribution

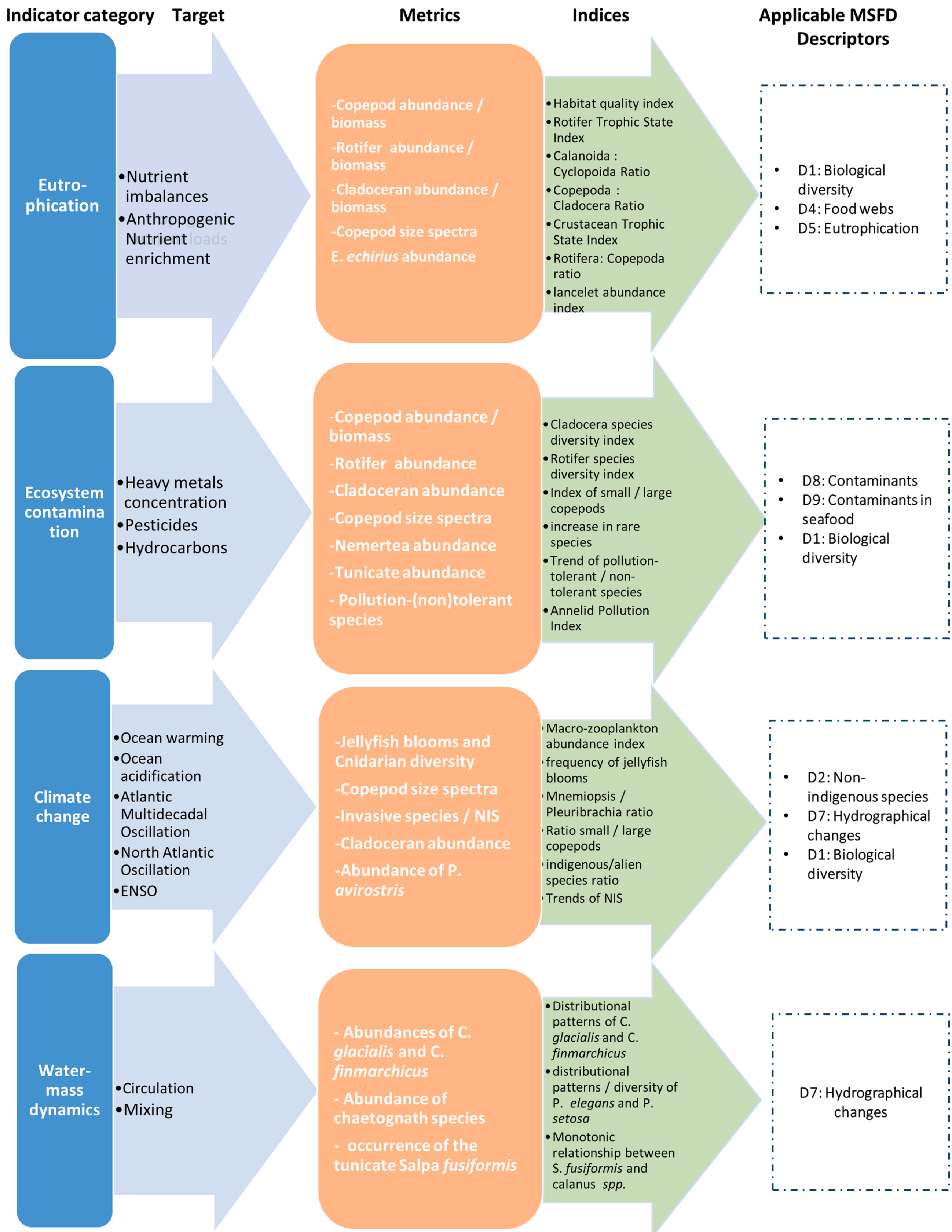

Fig. 4. Schematic of zooplankton-based pressure indices of eutrophication, contamination, climate change and water-mass dynamics.

of the lancelet populations (Vargas and Dean, 2010), implying that this taxon may be a sensitive indicator of nutrient enrichment and riverine sediment loading. Therefore, metrics based on the populations of adult cephalochordates may be relevant to Descriptors 5 and 6 on sea-bed integrity and eutrophication.

The individual zooplankton lifeforms mentioned in Fig. 4 can constitute a practical basis for marine trophic status indicators. In addition to individual lifeforms, a holistic 'biotic integrity' index comprising the use of entire communities or species assemblages can be used to capture a broad range of environmental conditions linked to eutrophication, in a multi-metric approach.

4.2.1.2. Zooplankton-based indices of water contamination. The contemporary intensification of human activities in the marine environment has occurred at the expense of water quality of which acute pollution or toxic contamination is of major concern. The main challenge of assessing contamination in the sea is that pollution sources and pathways are many and varied, consisting of point and non-point sources from agricultural, domestic, industrial effluents and/or river runoff including toxic chemicals, radionuclides, organic contaminants (POPs), trace metals (Law et al., 2010). For this reason, Descriptor 8 of the MSFD addresses the levels and effects of contaminants, and their spatial extent, in the marine environment, that can be negative to marine organisms, habitats and human wellbeing (D8: Concentrations of contaminants are at levels not giving rise to pollution effects) (Walmsley et al., 2016). Due to the difficulties of quantifying pollution stress and its impacts on the ecosystem, biological approaches have been suggested including the assessment of community structure, and measurements based on individual indicator species (Ismael and Dorgham, 2003). The latter is a pollution assessment approach that has stood the test of time, based on the rating of species according to their autoecology, referring to species' tolerance to different types and levels of pollution or toxicity (Patrick and Palavage, 1994). Therefore, rare species that proliferate suddenly with no known cause, or the sharp increase or decrease in known pollution-tolerant or sensitive species may qualify as good indicators of environmental contamination. Patrick and Palavage (1994) reviewed plankton indicators of water quality to assess the effectiveness of the Clean Water Act in improving the biological and chemical conditions of the Delaware and Neches Estuaries based on the ratio of pollution-tolerant species to the inherent characteristics of natural water conditions. A list of pollution-tolerant zooplankton indicator species courtesy of Patrick and Palavage (1994), relevant to marine environments, included the subclass: Malacostraca (Order: Isopoda, Amphipoda, Decapoda, Thoracica), the copepods Cyclopoida (*Paracyclops coronatus*) and Calanoida (*Pseudodiaptomus* spp. and *Acartia tonsa*), and Balanidae (*Balanus* sp., *B. balanoides* and *B. improvises*). Drira et al. (2018) also studied the spatial distribution of copepod assemblages in the Gulf of Gabes (Mediterranean Sea) to reveal their indicator value in response to physicochemical water quality stressors. The authors found that pollution-tolerant species including *Oithona nana, Paracalanus parvus, Harpacticus littoralis and Tisbe battagliai,* proliferated in shallow stations characterised by high coastal anthropogenic inputs where as high copepod diversity and large-size copepod species dominated by *Calanus helgolandicus* were prominent in the deeper offshore zone away from sewage pollution centers. Similarly, a sharp and sudden decline in copepod abundances was found to indicate the presence of toxic pollutants such as mercury, lead, copper, silver, cadmium, pesticides, and oxidants (chlorine and bromine) (Buchanan, 1993), that can propagate higher up in the food chain. For instance, an abrupt decline in the abundances of *Acartia tonsa* and *Eurytemora affinis* was observed in response to Tributyltin (TBT), a very toxic contaminant used as an antifouling agent in boat paints (Antizar-Ladislao, 2008). In contrast, Hypotrich ciliates, a group of ciliated protozoa, especially *Euplotes* spp. were found to increase rapidly in response to heavy metal contamination, hypoxic-anoxic conditions, and deficient dissolved oxygen levels (Buchanan, 1993), while abrupt changes in Nemertea and tunicate populations have been observed in response to heavy metal pollution (e.g., Papadopoulou and Kanias, 1977; Gibson et al., 1993). Surugiu (2005) identified pollution-tolerant and sensitive Polychaeta species serving as indicators of eutrophication and organic enrichment of the Black Sea. According to the author, tolerant opportunistic species included *Capitella capitate, Polydora cornuta, Heteromastus filiformis, Lagis koren, Melinna palmate, Neanthes succinea, Harmothoe imbruicata, and Prionospio cirrifera.* Non-tolerant Polychaeta species that respond to increased levels of organic pollution by drastically reducing their abundances, including members of the genus *Syllis*, *Perinereis cultrifera, Nereis zonata, Syllis gracilis, Typosyllis hyaline, Nereiphylla rubiginosa* and *Micronephthys stammeri, Ophelia bicornis, O. limacina, Pisione remota, Polygordius* sp., *Glycera convoluta, Nephtys cirrosa, Magelona rosea* and *M. papilicornis.* Based on the polychaete metrics, an 'Annelid Pollution Index (IPA)' was proposed as ratio of the best polychaete sentinels of polluted water to the pollution sensitive or polychaete sentinels of clean water (Surugiu, 2005).

Barnacles in the British waters including *Balanus* (*Amphibalanus*) *improvises, B. amphitrite* (*Amphibalanus Amphitrite*) and the invasive species *Austrominius modestus* were also described as good pollution-tolerant indicator organisms capable of thriving in highly eutrophic waters and under conditions of chemical / organic pollution (Crisp, 1958). Moreover, ecologically important ubiquitous crustaceans such as mysids that link the benthic and pelagic systems (Anderson and Phillips, 2016) indicated water quality and benthic-pelagic coupling due to their high sensitivity to changes in salinity, turbidity, water circulation and chemical contamination from heavy metals (USEPA, 1972; USEPA, 1996; Roast et al., 1998; Anderson and Phillips, 2016). Individual lifeforms or community assemblages have therefore been shown to respond to specific environmental health issues, based on their sensitivities to known stressors. Taxa that are sensitive to known contaminants are potentially reliable bio-indicators capable of serving as early warning signals for acute pollution applicable to Descriptors 8 and 9 of the MSFD (referring to the presence and levels of toxic contaminants in European waters) (Fig. 4). Such metrics must however undergo stringent tests in the context of the MSFD to establish their indicator value for marine ecological status assessments in different regions. Descriptor 8, dealing with the effects of contaminants and chemical pollution is closely linked with Descriptor 5 (nutrients), Descriptor 9 (contaminant concentrations in marine species and seafood), and Descriptor 10 (marine litter), all of which have potentially adverse impacts on biodiversity (Descriptor 1), the integrity of food webs (Descriptor 4) and sea-floor integrity (Descriptor 6). The metrics and indices of environmental contamination identified above may therefore also apply to the related Descriptors as secondary indicators.

4.2.2. Indicators of ocean-climatic changes

The world's oceans and seas and their various ecosystems are increasingly under the influence of anthropogenic climate change occurring at different temporal scales simultaneously with the background natural variability (Bedford et al., 2019). Consequently, the MSFD seeks to ensure that anthropogenic activities do not permanently alter hydrographical conditions (e.g. temperature, salinity, depth, currents, waves, turbulence, pH, pCO_2), that can have major adverse effects on the dynamic functioning of marine ecosystems and lifeforms (Zampoukas et al., 2014). This is captured in Descriptor 7 of the MSFD (D7: Permanent alteration of hydrographical conditions does not adversely affect marine ecosystems). According to the 2016 Guidance for the Marine Strategy Framework Directive assessments, the two main criteria for assessing D7 include the 'spatial extent and distribution of alterations in hydrographical conditions (D7C1)' and the 'spatial extent of adverse effects on benthic habitats from permanent alteration of hydrographical conditions (D7C2)'. Planktonic communities, described as 'sensitive beacons of climate change' (Richardson and Gibbons, 2008), are central to the characterization and monitoring of the ecological impacts due to climate change (Reid and Edwards, 2001; Beaugrand et al., 2004; Beaugrand, 2005; Benedetti et al., 2019). For instance, egg viability and nauplii development in the calanoid copepod *Acartia bifilosa* (Vehmaa et al., 2013), and the abundance and body size of calanoid copepods *Acartia* spp., *Centropages* spp., *Pseudocalanus* spp. and *Paracalanus* spp., decreased sharply in response to the effects of ocean warming and acidification (Garzke, 2014; Garzke et al., 2014). Some authors have also hypothesized drastic changes in zooplankton community structure in response to climate change such as the future establishment of temperate Cirripedia species in the Arctic (Walczynska et al., 2019) and shifts in plankton communities toward smaller cells (Makinen et al., 2017), reduced carbon export rates and the increased roles of gelatinous zooplankton (Troedsson et al., 2013). The significant shifts in zooplankton community structure and size-spectra towards the dominance of the small-sized copepod *Oithona similis* relative to large-bodied calanoid copepods have already been observed across the global ocean such as in the Arctic (Balazy et al., 2021), the North Sea (Torkel and Sabatini, 1996; Bedford et al., 2019), the North Atlantic and the Mediterranean Sea (Beaugrand et al., 2004; Beaugrand, 2005; Goberville et al., 2013; Castellani et al., 2016). The dominance of *O. similis* reflects the species' high thermal tolerance, flexibility and adaptive capacity, explaining why the species thrives under conditions, which are thermally unfavourable to other species, including a wide range of temperatures from < 0 °C (Balazy et al., 2021) up to ~ 17 °C(Castellani et al., 2007; Castellani et al., 2016). For these reasons, temporal and spatial changes in the biomass and other functional traits of Calanoida relative to the more resilient *O. similis* can indicate underlying effects of climatic changes on marine ecosystems. Hence, indices such as the ratio of *O. similis* relative to calanoid copepods and the biomass or trend of Cirripedia can constitute strong indicators of fundamental ecological and biodiversity changes linked to anthropological and climatic alterations. These can be linked directly to the biodiversity and hydrography descriptors of the MSFD (D1 and D7).

Furthermore, many researchers have attributed the increasing presence of non-indigenous species such as the invasive ctenophore *Mnemiopsis leidyi* (Hansson, 2006; Bolte, 2013; Granhag and Hosia, 2015; Vansteenbrugge et al., 2016) and the abrupt appearance of the cladoceran *Penilia avirostris* (Johns et al., 2005) and the invasive calanoid copepod *Pseudodiaptomus marinus* (Brylinski et al., 2012; Jha et al., 2013; Deschutter et al., 2018; Seregin and Popova, 2020) in the North Sea and other European seas, to climate-induced warming. The Convention on Biological Diversity recognizes the presence of non-indigenous species (NIS) as the second most significant threat to global biodiversity (European Environmental Agency, 2012) and thus a challenge to achieving good environmental status in EU waters (European Commission, 2010) and globally.

Moreover, Smith et al. (2017) found via experimental field research that the pontellid copepod, *Labidocera* spp. is highly sensitive to ocean acidification, decreasing by up to 70 % in response to increased CO_2 conditions. In contrast, although appendicularians did not appear to have a significant indicator value as eutrophication indicators (de Carvalho et al., 2016), mesocosm experiments revealed that the appendicularian *Oikopleura dioica* increased substantially in response to rising pCO_2 levels, temperature rise and reduced *pH* (Troedsson et al., 2013; Bouquet et al., 2018), making them potentially strong indicators of climate-related oceanographic changes. This implies that increasing populations of appendicularians in the water column could indicate rising organic carbon concentrations, pCO_2 levels and declining *pH*, which may be detrimental to overall biodiversity and ecosystem health.

Therefore, metrics such as trends in the abundance of appendicularians, the introduction rates and spatial distribution NIS, the total of all new NIS observed in a given area, the success rate of their colonization in terms of their ability or inability to establish self-sustaining populations (Teixeira et al., 2014; Teixeira et al., 2016; AquaNIS. Editorial Board, 2015; Olenin et al., 2016), the cumulative number of alien species since 1900, and invasive alien species threatening biodiversity (European Commission, 2010, European Environmental Agency, 2012), can directly indicate broad marine ecological changes in response to climate-related and anthropogenic alterations.

Based on this information, we can derive and test several indices in the North Sea, including the ratio of *M. leidyi* to autochthonous ctenophores (e.g. *Pleurobrachia pileus*) and the ratio of *P. avirostris* to the native *Evadne* spp. The frequency and magnitude of gelatinous zooplankton blooms have also been linked to rising temperatures (Brodeur et al., 1999; Lynam et al., 2004, 2005; Gibbons and Richardson, 2009; Qu et al., 2014), ocean acidification (Attrill et al., 2007) and global climatic oscillations (Condon et al., 2013) and thus can serve as a reliable index of ecosystem structural change in response to climate-related alterations, amidst considerable persistent debate on the subject (Condon et al., 2012; Pitt et al., 2018). These indices and metrics can be used to infer future ecological stressors if factors favouring current invasions and blooms are known and are directly applicable to Descriptor 2 of the MSFD (D2: ecological threats by non-indigenous species on natural biological diversity and D7: hydrographical changes). The pressure and impacts of NIS are related directly to Descriptor 2, Criteria 1 and 2 (D2C1C3) (Fig. 4).

4.2.3. Indicators of water-mass dynamics

Changes in water mass structure including water mass stability, salinity gradients, the position of tidal fronts, and associated stratification / de-stratification processes, caused by either climate-related or human-induced alterations (Tian et al., 2011) have significant effects on marine ecological status. These dynamic hydrographic patterns potentially drive changes in marine primary productivity and influence planktonic distribution over time and space. Consequently, specific zooplankton taxa used as tracers of water-mass interactions, mixing and other important transient features (Meek, 1928; Russell, 1935a; Russell, 1935b; Russell, 1936; Choquet et al., 2018) can effectively indicate major underlying hydro-climatic changes and their related ecological impacts. For instance, changes in the abundance and distribution of the copepods *Calanus glacialis* and *C. finmarchicus* in the Arctic and Atlantic Oceans, respectively, are often indicative of climate-induced changes in water mass interactions between both oceans (Choquet et al., 2018). Metrics such as the distributional patterns and diversity of chaetognath species *Sagitta elegans* and *Sagitta setosa* across the North Sea have also been attributed to water-mass interactions between the North Sea and North Atlantic waters (Meek, 1928; Russell, 1935b; Russell, 1936; Bone et al., 1987), and in the Pacific (Bieri, 1959). These Chaetognatha species are therefore important indicators of water mass changes due to their preference of specific salinity and temperature conditions. Chaetognaths have also been found to influence different levels of the trophic chain and the movement of and recruitment of herring (Savage and Hardy, 1935). Subsequent studies successfully linked the abundance of fish eggs and larvae in the northern North Sea to the alternating dominance *S. elegans and S. setosa*, with the former coinciding with the high abundance of the larvae of certain fish species than when latter species was present (e.g. Bone et al., 1987). The reason for this is likely because chaetognaths are voracious predators feeding primarily on fish larvae and crustaceans thus exerting tremendous pressure on fish through direct predation or competition for food (Every, 1968). Hence, the cyclicity of chaetognath species can also serve as an early-warning indicator for fish larvae survival and recruitment. This is highly relevant to Descriptors D3 (fish stocks) and D4 (food-webs) because fish larvae are important top-down regulators of the plankton ecosystem and fish stock replenishment (Lancaster, 2006). Hence, fish egg/larvae abundance, distribution and responses to stressors must be understood and closely monitored for effective fish stock assessments, biodiversity assessments and sustainable ecosystem management.

Furthermore, the periodic occurrence of the tunicate *Salpa fusiformis* (a planktonic herbivore) in the North Sea and the simultaneous decline in the abundance of *Calanus* spp. (Tams-Lyche, 1966) have been linked to inflows of oceanic waters. Similarly, the appearance of rare zooplankton species in the Baltic Sea about three decades ago was attributed to saline water influx from the North Sea (Radziejewska et al., 1973). Wasmund et al. (2016) also observed the increase in the overall abundance of zooplankton taxa related to the inflow of saline water to the Baltic Sea, with halophilic species such as *Acartia clausi, Calanus* spp.*, Oithona atlantica*, *Penilia avirostris* (Cladocera) *and S. setosa* (Chaetognatha), serving as potential indicators of saline water intrusion.

Another taxon heralded as a potential indicator of oceanographic change is Appendicularia. For instance, in the Drake Passage, Kalarus and Panasiuk (2021), found that temperature and salinity were the strongest environmental factors influencing the larvacean community structure. According to the authors, the most abundant species *Fritillaria borealis* was more ubiquitous in space, while the distributions of *Fritillaria fraudax* and *Oikopleura gaussica* were limited to specific hydrological zones and conditions in frontal areas, and correlated with temperature and salinity. Furthermore, Flores-Coto et al. (2010) analyzed the mesoscale spatial distribution of *Oikopleura* and *Fritillaria* in the southern Gulf of Mexico where the temperature was not a limiting factor and found that water turbidity was the main factor affecting the spatial distribution of both genera. The authors revealed that *Fritillaria* was dominant in turbid waters off the main fluvial-lagoon systems while *Oikopleura* was the most abundant in upwelling areas, likely due to its broad diet and higher filtration efficiency for larger particles allowing it to exploit the primary production in highly energetic upwelling areas (Flores-Coto et al., 2010) and frontal zones (Kalarus and Panasiuk, 2021). Water masses along the coastal – offshore gradient of the Gulf of Mexico were also shown to be better differentiated by the occurrence of certain pteropods (Austin, 1971) and cephalopods (Voss, 1967; Lipka, 1975) than by physicochemical parameters. Hence, pteropods such as holoplanktonic molluscs were identified as potential indicators of offshore water intrusion (Austin, 1971) while the distribution of pelagic cephalopods indicated vertical water structures (Voss, 1967; Lipka, 1975). Marine copepods, vital for the sustenance of the marine food web were also recognized as strong bioindicators of ocean hydrographic changes several decades ago. As early as the 1950s, Fleminger (1957, 1959) identified pontellid copepods as potential indicator species of different water masses in the Gulf of Mexico. The authors described *Labidocera acutifrons* and *Pontella spiniceps* as strong indicators of tropical-oceanic waters, *Labidocera aestiva* and *Pontella meadi* as temperate – neritic indicators, *Labidocera scotti* as a tropical-neritic indicator species and *Centropages hamatus, Acartia tonsa and Pseudodiaptomus coronatus* as indicators of temperate – neritic North Atlantic waters. Therefore, metrics based on the above species distribution and density can potentially indicate changing oceanographic conditions induced by vertical and horizontal water mass interactions and can serve as early warning signals of more fundamental changes that would otherwise be difficult to detect. Based on these examples, demographic metrics derived from specific zooplankton lifeforms that effectively track water mass patterns and movements can be used to develop indices associated with MSFD D7.

5. Discussion

The scale of environmental and anthropogenic pressures in the world's oceans and seas is increasing, threatening marine ecological sustainability and human socio-economic well-being. The complexity associated with protecting marine ecosystems from multiple simultaneous stressors while continuing to reap the benefits (ecosystem goods and services) offered by these highly dynamic but fragile environments requires a paradigm shift from the fragmented sectoral approach to an ecosystem-based management approach. The MSFD embodies this new paradigm by introducing a holistic approach to all aspects of ecological assessment, monitoring and management through legislation. An efficient, cost-effective and robust way of diagnosing environmental status is the use of quantitative ecological indicators and indices as mandated by the European Union through the MSFD (Niemi and McDonald, 2004). In this study, we undertook a review of bio-indicators and catalogued a variety of zooplankton-based indices using a newly developed IMIC to facilitate the implementation of the MSFD. Our main finding based on the literature is that there has been much progress in the development and use of holistic indices for assessing and monitoring broad ecosystem structural and functional changes directly applicable to the Biodiversity and Food web Descriptors (D1 and D4), but these are limited in their ability to inform on the specific drivers or pressures. We, therefore, put greater emphasis on zooplankton pressure indices because these have not been explicitly developed or tested in the context of the MSFD. The reason for this per the MSFD Common Implementation Strategy (2017) is that the effective management of marine environmental pressures can accelerate the process of recovery towards GES. For this reason, pressure indices were largely derived from the global literature addressing the responses of individual zooplankton communities or species to specific stressors. Summary tables of existing and proposed metrics and indices are provided in Annex 1 and 2 (Fig. 4). We recognize that despite years of progress in the context of the MSFD, only a few zooplankton indices have been exclusively linked to specific stressors and descriptors, and major issues related to indices development, application and interpretation persist.

5.1. General challenges of zooplankton indices development and application

The development and application of zooplankton-based indices face several challenges. Over-simplification is an issue common to the development and use of all indices. The abstraction of complex multivariate ecological responses to single-values (Buchanan, 1993) implies the loss of vital information, resulting in only a partial representation of system state and dynamics that can lead to erroneous diagnoses of ecological problems. According to our assessment, some of the major issues related to the development and application of indices include:

5.1.1. The complexity of pressure indices

A major challenge of the MSFD implementation is that environmental stressors have not been effectively incorporated into the assessment of GES due to the complexity of the relationships between the hydrographic conditions and the biotic component of the marine ecosystem. Descriptor 7, for instance, focuses on permanent alterations of hydrographical conditions by anthropogenic activities, with adverse impacts on the dynamic functioning of marine ecosystems (Zampoukas et al., 2014), but no generally agreed definition of 'permanency' exists. HELCOM however suggests an arbitrary period greater than ten years. Moreover, information on existing pressure indices and their calculation methods are scattered throughout the vast literature and thus not readily available to researchers. Therefore, this study fills this gap by identifying and synthesizing a range of zooplankton-based pressure response metrics and indices applicable to Descriptors D2, D5, D7 and D8. Such pressure indices can effectively complement the holistic biodiversity indices by highlighting the ecological responses to anthropogenic stressors.

5.1.2. Lack of reliable reference conditions

To develop GES indices, the quantitative definition of thresholds or reference periods is imperative to determine if and when the good environmental status is achieved. Five methods are currently used to determine reference periods. These include the use of, i). Comparable, currently minimally impacted marine regions as a reference, ii). Historical records of minimal impact at a given site, iii). Modelled reference values (e.g. results of state pressure modelling or hindcasting), iv). Defined baselines based on an agreed reference variable of a current or past state), and v). Expert judgements on suitable reference points in the case where historical, current and modelled reference values are not available (German Federal Agency for Nature Conservation and the Federal Environment Agency, 2011). However, the application of the most reliable methods (i– iv) within the MSFD remains elusive (Tam et al., 2017) because most hydrographic and biological time-series data series are not long enough for relevant historical marine reference conditions to be defined. Moreover, the non-existence of near-pristine marine environments, coupled with the high degree of unpredictability of zooplankton communities hinders the definition of the baselines necessary for the identification of a threshold value for GES (Caroppo et al., 2013). A study by Bedford et al. (2019) revealed that the changes observed in the North Sea ecosystem today had already been ongoing since the beginning of the 20th Century. Consequently, reference periods are currently only arbitrarily defined, mainly based on expert knowledge and/or some assumed historical reference points. Some researchers have proposed a shifting baseline approach under the rationale that the use of multiple temporal scales can unveil information relevant to detecting the multi-temporal fingerprint of climate change relevant for marine environmental management (Bedford et al., 2020). While this method has its merits in that it can assess the recent state of the ecosystem as well as its flexibility to accommodate different policy questions, it still does provide information on past states of the ecosystem, thus leaving the question of what constitutes a good ecological status unanswered.

5.1.3. Index circularity

The previous issue also raises another unresolved challenge: index circularity, associated with the development of pressure indices that stem from the use of data from within a system to define reference conditions and develop indices to assess the said system. Hence, pressure indices as currently implied in the literature, lack a sufficient theoretical background due to the novelty of the ecological indicator approach and limited long-term data necessary for defining reference points. As suggested by HELCOM, neighbouring areas for which extended datasets are available can be preferably used as reference sites (e.g. HELCOM, 2015, 2018) to overcome the risk of circularity. The issue of circularity can also be overcome by first identifying indicator taxa or species, detecting their temporal variability patterns, and then finally determining the hydrographic and environmental stressors responsible for the observed patterns, rather than the other way round.

5.1.4. Index ambiguity

Index ambiguity characterizes most of the existing holistic and pressure indices. It implies that pressure indices do not usually infer specific stressors but rather apply to a broad range of pressures. For instance, the abundance and body size of calanoid copepods can indicate both the effects of ocean warming/acidification (Garzke et al., 2014; Alguero-Muñiz et al., 2017) and eutrophication (Margoński et al., 2007; Gorokhova et al., 2013; Thompson et al., 2013; HELCOM, 2013b; HELCOM, 2018; Simm et al., 2014; Labuce et al., 2020). Therefore, most indices do not sufficiently inform on the specific effects of specific stressors and hence do not have a substantial indicator value for these stressors.

5.1.5. Index comparability

Furthermore, the MSFD aims to achieve a comparable level of GES in marine waters in the EU via harmonized and comparable data collection programs and schemes, but index comparability between EU member states remains a major challenge. This is because of differences in abiotic and biotic conditions and different sampling strategies across different countries (Zampoukas et al., 2014). Hence, it is less likely to directly apply or compare pressure indices from one country to another. Only a limited number of holistic indices of biodiversity and food-web structure, including the MSTS, PLI, and the metrics such as arrival rate of NIS, have *trans*-regional applicability, but again, these are limited by their inability to effectively infer specific ecological stressors.

5.2. Some proposed solutions

To overcome some of the salient issues such as index ambiguity, Berger et al. (2018) recommended extracting key biological and functional traits from indicator species and taxa and using these to achieve higher stressor specificity. We posit that the future success of zooplankton indices and bio-indicators, in general, depends on our ability to choose the best indicator species and optimally use these to infer specific environmental conditions, based on advanced statistical and modelling techniques, validated by laboratory experimentation and monitoring data. Another step in the right direction would be to heed the recommendation of prominent researchers in the bird indicator community e.g. Buckland et al. (2005) and van Strien et al. (2012), to use composite indices developed from trends of indicator species or communities (sensitive to environmental change over relatively short timescales) preferably based on the geometric mean of relative abundances. The advantage of such indices is that they can be further linked with the causes of the observed trends (van Strien et al., 2012). The success of these approaches requires region-specific assessments of environmental status followed by the standardization of appropriate indices that effectively integrate the effects of multiple stressors taking into consideration regional/local specificities. Hence, since the numerous indices described in this study were derived from a variety of sources worldwide, they may not automatically apply in every marine region. For instance, indices developed in the North Sea based largely on the dominant community copepods, representing North Sea conditions and environmental status may not apply to the Baltic Sea dominated by rotifers, cladocerans and small-bodied copepods. This implies that the development and application of specific indices should be based on a good fore-knowledge of the area in which the index was developed.

As a practical example, we describe here a region-specific example in the German North Sea (GNS) (work in progress) in which we applied the strategies described in this study to develop state and pressure indices using zooplankton lifeforms. We started by identifying reliable indicator species including sensitive and tolerant lifeforms and their response to cumulative or specific environmental stressors using robust statistical methods. Finally, we used species or lifeform pairs with strongly opposing inter-annual variability patterns to develop indices for describing the general ecological state as well as inferring specific ecological stressors. For instance, a high Bryozoa to Spionidae ratio was found to potentially indicate eutrophication (D5); a high ratio of shell-formers to appendicularians and/or cephalochordates indicated the chemical pollution status (D8). The inverse to the latter index was also potentially indicative of ocean acidification/warming (D7), while the trend or ratio of rare and alien species (e.g. *P. avirostris and P. marinus*) relative to native and common ones was proposed as D2 indicators.

Finally, we highlight here the need for index standardization since indices developed from different assessment programs may not be directly comparable. Standardizing national ecological indices can be achieved by the joint effort of marine ecologists, physical oceanographers, climatologists, statisticians, data scientists, policymakers and other relevant stakeholders. National level standardization should logically be followed by regional level standardization achieved by all relevant stakeholders from across the EU, based on mutually agreed protocols to derive ecologically and policy-relevant, user-driven, easily understood practically feasible and comparable indices. These recommendations could make a considerable contribution to the field of marine ecological indicators development and application in general. The expected improvements in the quantitative and mathematical robustness of the zooplankton indicator system and their communication through various scientific media will also greatly facilitate the implementation of the MSFD. Such an improved zooplankton indicator system may also be adopted into the suit of biodiversity indicators for monitoring Global Biodiversity Targets per the UN Strategic Plan for Biodiversity (https://www.cbd.int/sp/targets/) that until recently had a strong bias towards terrestrial systems.

6. Conclusion

In this study, we recognized the immense potential for using zooplankton in ecological assessments in the context of the MSFD. To enhance the understanding and application of zooplankton indices, we introduced a comprehensive iterative framework known as the IMIC to highlighted the fact that indicators, metrics and indices are interlinked but not interchangeable components of an inclusive ecological indicator system. Using this framework, we classified the existing indicators and related indices into two broad categories. The holistic biomass-based indices (including the mean size and total stock and the plankton life-form index) captured essential changes in ecosystem structure and functioning, and the stressor-response indices – targeted at specific issues linked to the local effects of climate and anthropogenic pressures. The IMIC revealed that together, the holistic and pressure-specific categories of zooplankton indices form a spectrum of indices that can foster the implementation of the MSFD based on the 11 GES descriptors. For this reason, we suggested that holistic indices be supplemented with carefully selected area-specific stressor-response indices to fulfil the technical properties of a robust ecological indicator system. Finally, we identified the main limitations of holistic indices linked to their ambiguous nature, and the range of challenges associated with developing, applying and interpreting pressure indices. Prominent among these were problems of index ambiguity, circularity and the non-existence of appropriate reference periods or sites, which limit indicator efficiency and appeal. These issues highlighted the need for future improvements of the ecological indicator system to transform zooplankton indices into standard tools for achieving marine environmental assessment targets. We recognize that the field of ecological indicator development and application is relatively young and growing, and stands to benefit immensely from the rapid progress in multivariate statistics and ecological modelling. There is also the opportunity to borrow from other fields or communities, such as the 'bird indicator' community, which itself relies on econometric theories such as the 'price index theory', for the fundamentals of their indicator development approaches. Our study, therefore, serves as an information source for scientists, environmental managers, and policymakers interested in developing and using zooplankton indices to implement the MSFD in European waters and globally.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2022.108587.

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