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STATE OF THE ART AND PERSPECTIVES ON THE USE OF PLANKTONIC COMMUNITIES AS INDICATORS OF ENVIRONMENTAL STATUS IN RELATION TO THE EU MARINE STRATEGY FRAMEWORK DIRECTIVE

STATO DELL'ARTE E PROSPETTIVE NELL'UTILIZZO DELLE COMUNITÀ PLANCTONICHE COME INDICATORI DI STATO AMBIENTALE SECONDO LA DIRETTIVA QUADRO EUROPEA SULLA STRATEGIA MARINA

Abstract - *Planktonic communities hold a relevant role in the framework of the EU Marine Strategy Framework Directive. This paper summarizes the current state of art in the use of plankton as indicator for the assessment of the environmental status of marine environments, contributing to the discussion of new perspectives in its application for the implementation of the Directive.*

Key-words: *bacteria, phytoplankton, zooplankton, Marine Strategy Framework Directive.*

Introduction - The Marine Strategy Framework Directive (2008/56/EC, hereafter MSFD) has been developed with the overall aim of promoting sustainable use of the seas and conserving marine ecosystems. It represents the response to concerns that existing legislation for the protection of the sea from some specific impacts was too sectorial and fragmented. In the MSFD there is recognition that the diverse conditions, problems and needs of the various marine regions or subregions in the Community require different and specific solutions. Member States (MS) are therefore working on the 'building blocks' leading to the preparation and planning of measures to achieve Good Environmental Status (GES) at the level of marine regions or subregions. The 'building blocks' of the MSFD are: i) the assessment of essential features and characteristics, and of predominant pressures and impacts; ii) the determination of GES for 11 qualitative descriptors by using a set of criteria and indicators; iii) the establishment of Environmental Targets and associated indicators so as to guide progress towards achieving GES in the marine environment; iv) the establishment and implementation of coordinated monitoring programmes for the ongoing assessment of the environmental status of their marine waters. Planktonic communities need to be taken into consideration in different descriptors of the MSFD, namely D1 (Biological Diversity), D4 (Food Webs) and D5 (Eutrophication). In this paper we refer only on Biological Diversity and Eutrophication.

The EU Reporting of the initial assessment (Art. 8 of MSFD) has been carried out by each MS at the level of 'assessment areas' (AAs). In our national report to the EU, AAs were slight different for the two descriptors and for Microbial Pathogens, in relation to the available data referred to the period 2007-2011. For each AA an analysis of pressures, impacts and activities was carried out, according to the DPSIR procedure.

Substantial work is still needed to clearly define the use of planktonic communities as indicators for the assessment of GES due to the lack of data with adequate spatial and temporal coverage and lack of established methods at the regional and/or subregional level. The aim of this paper is to describe the potential use of planktonic communities as indicators of environmental status and the perspectives to better define the functionality of the ecosystem and its quality conditions.

Phytoplankton

Phytoplankton biomass, mainly in terms of chlorophyll *a* concentrations, was used as an indicator of trophic conditions already in the 1960s. Later on, the need of assessing trophic status of aquatic ecosystems became a priority worldwide due to the serious impacts caused by eutrophication phenomena. Chlorophyll *a* concentration became the most commonly and routinely used indicator of trophic conditions, being easily measurable and well-correlated with nutrient enrichment (i.e. Ferreira *et al*., 2011 and references therein). The Water Framework Directive (WFD - Directive 2000/60/EC) and the marine conventions (OSPAR, HELCOM and Barcelona Convention) require the use of phytoplankton to assess water quality, and promoted and addressed several approaches on the use of various metrics beyond chlorophyll concentration, such as cell abundance, biomass as carbon content, cell size, diversity, etc. (Facca *et al*., 2011; Lugoli *et al*., 2012; Garmendia *et al*., 2013). The WFD, in particular, explicitly requires the assessment of ecological status of coastal and transitional waters based on the Biological Quality Element (BQE) ‘Phytoplankton’, which is considered in terms of abundance and species composition. The EU and MS have set up intercalibration exercises of BQEs within the WFD (MED-GIG), in order to achieve a coherent approach in the classification of environmental quality among Mediterranean countries. So far, we did not reach a consensus for coherent and shared view at the Mediterranean level for the BWE ‘Phytoplankton’. Most of the countries’ proposals use only chlorophyll concentrations and/or bloom frequency and the main weakness is related to the lack of reliable taxonomic information useful for the description of community composition and of diversity index calculation. Some interesting results were achieved in the area with a good expertise in taxonomic studies (Facca *et al*., 2011; Bazzoni *et al*., 2013), but further work is needed and a Commission Decision on this specific BQE is expected by 2015 after the completion of a third intercalibration exercise. MSFD and WFD have comparable objectives and some significant areas of overlapping (i.e. in relation to eutrophication and ecological quality) but they have different approaches: WFD bases the overall status on the worst result among the four BWE (one-out-all-out approach), whereas the MSFD approach is more holistic and aware of the role of functional aspects (cfr. http://archive.defra.gov.uk/environment/marine/documents/legislation/msfd-factsheet1-waterdirective.pdf).

Likewise, although phytoplankton could be considered as an indicator for the evaluation of GES in several MSFD Descriptors (cfr. Commission Decision 2010/477/EU), a quantitative approach on how such indicator contributes to the definition of GES has yet to be determined and is expected to be finalized by 2018. In order to achieve that, it has been proposed to combine chlorophyll *a* measurements with the study of shifts in community composition (relative abundance of diatoms, flagellates, dinoflagellates, etc.) and possibly the presence of harmful species correlated to nutrient enrichments or other anthropogenic pressures. The compelling requirements of bio-monitoring (high frequency, large scale and long time series) could be highly facilitated by optical detection of blooms both through remote and *in situ*, active and passive measurements (Zampoukas *et al*., 2012) as they allow not just bulk assessment, but also functional types discrimination.

The use of sea water bio-optical properties both *in situ* measured and remotely sensed has been stated since decades as a powerful tool for phytoplankton distribution assessment. Recently the high spatial extension and temporal follow-up of remotely sensed data has been used also for water quality classification and with operative purposes. The reliability of monitoring through ocean color remote sensing is strictly dependent on locally validated algorithms, such as the one available for the Mediterranean Sea (Volpe *et al*., 2012). Thus optical Remote Sensing (RS) and surveys are really complementary and *in situ* measurements are required for optical components determination (Lapucci *et al*., 2012) as well as for optical properties, like absorption and fluorescence (Massi and Lazzara, 2010).

Spectral variations of sea water Optical Properties such as reflectance, absorption, backscattering and fluorescence can lead to the assessment of several "ocean color products" not just chlorophyll concentrations (CHL), but also of total suspended matter (TSM), water transparency (vertical diffuse attenuation coefficient Kd) and colored dissolved organic matter (CDOM) (Maselli *et al*., 2009). In fact, sea water optical components concentrations of CHL, CDOM, TSM can be obtained through inversion algorithms from sea-water Apparent Optical Properties (AOPs).

These are measured by optical sensors which can also be carried by autonomous devices (gliders, Argo floats), dispensable profilers and miniaturized systems (Xing *et al*., 2011; Nourisson *et al*., 2013) as part of monitoring programs of regional waters (Zampoukas *et al*., 2012). Further basic indicators of eutrophication, such as primary production, phytoplankton blooms and composition (Ferreira *et al*., 2011) can also be achieved through optical RS. Estimates of integrated primary production are extensively attained through the application to remote sensed data of bio-optical models (Lazzara *et al*., 2010; Uitz *et al*., 2012). Statistical metrics (phase, duration, frequency) of phytoplankton blooms is accessible from ocean color RS for eutrophication as well as for pelagic ecology and food web studies (Platt and Sathyendranath, 2008). Also, four to five phytoplankton functional types and three size classes have been detected from satellite at the global scale with increasing success (Nair *et al*., 2008), but still difficulties must be overcome in optically complex coastal waters and at the regional scale. Finally, the detection and monitoring of harmful blooms and of toxic species such as *Karenia brevis* has been obtained with use of optical RS (Stumpf *et al*., 2009).

In recent years, HPLC has been used to estimate phytoplankton composition by identifying photosynthetic pigments and include rapid turnover and reproducible results (Wright *et al*., 1996; Jeffrey *et al*., 1997). The method is based on analysis of accessory pigments, in addition to chlorophyll *a* (Chl *a*) or the modified divinyl-Chl *a* found in all phytoplankton species, and that some of these accessory pigments are specific for the individual phytoplankton groups (Millie *et al*., 1997; Wright and Jeffrey, 2006; Brunet and Mangoni, 2010; Mangoni *et al*., 2011). The few HPLC-based studies performed on phytoplankton size fractions revealing that this approach can provide insights into the taxonomic diversity and the physiological state of the small phytoplankton groups, which are normally unrecognizable by light microscopy and are often difficult to preserve (Andersen *et al*., 1996; Ansotegui *et al*., 2003).

Other indicators have recently been proposed to evaluate the potential of the use of phytoplankton as indicator to discriminate between pristine and disturbed marine systems, and that are based both on size structure and functional attributes (autotrophs *vs.* heterotrophs) (see Garmendia *et al*., 2013 for a review).

Zooplankton

The response of zooplankton to environmental conditions is of particular interest due to the central role that this group occupies as a trophic link between planktonic primary producers and larger consumers. Any variation in zooplanktonic biomass has implications on biogeochemical cycling, trophodynamics, fisheries and ecosystems services (e.g. target organisms are important trophic links to many commercially and recreationally important species).

It is well know that zooplankton composition and abundance are influenced by the water masses properties and in coastal as well as pelagic environments, changes in communities structure can indicate potential damages induced by natural or anthropogenic impact. In fact, during the last decades, zooplankton communities in different areas underwent a substantial transformation, including changes in abundance and phenology in the majority of the species, increase of smaller species, etc. (Hays *et al.*, 2005; Conversi *et al.*, 2010).

In general, there is an uniformity in zooplankton communities structure with seasonal changes of peculiar species association. The persistence of recurrent patterns in species distribution makes it very unlikely that a purely stochastic process drives the plankton community structure (Kruk *et al.*, 2002). Nevertheless, the zooplankton community is characterized by a pronounced degree of unpredictability that hinders the definition of the "baselines" necessary to identify a "Threshold Value" for the definition of GES of the marine environment.

More information on the zooplankton communities, including the species composition/distribution and seasonal/geographical variability, can provide a relevant contribution to the definition of GES for various Marine Strategy Framework Directive (MSFD) Descriptors. GES zooplankton indicators can be classified into different categories ranging from rather reductionistic to holistic indicators that integrate a broad range of environmental information (Burkhard *et al.*, 2008) and are more generally related to the concepts of ecosystem health and integrity. For example: i) indicators based on the abundance of selected species (direct measurement); ii) indicators based on matching species and samples, commonly in use to define species-sample and relation between different classes of organisms or recurrent groups, site or season (e.g. Recurrent group analysis – RGA, Correspondence analysis – CA, etc); iii) indicators based on ecosystem composition and structure (the very commonly calculated Shannon-Wiever index, more sensitive to rare species, or the Simpson's index that puts weight on the common species).

Recently, a species-level approach has been recommended for plankton studies. Such an approach is aimed to improve the understanding of community dynamics and biological interactions, and to the developing of predictive modeling capability (Marine Zooplankton Colloquium 2, 2001).

Comparative ecosystem responses among contiguous ecosystems can reveal whether common, large-scale forcing drives common ecosystem responses (Alheit *et al.*, 2005). Common indicator/parameter set, based on a broad enough common conceptual ground, allow to cover multiple aspects of the ecosystems. In this context the Long Term Ecological Research (LTER) represents a worldwide efforts to better understand the modes of zooplankton populations and community structure and the evolution at different temporal scales (Mirtl *et al.*, 2009). This comprises the study of their structure, functions, and response to environmental, societal and economic drivers as well as the development of management options.

In the Mediterranean Sea, within the LTER network, five ongoing time-series are monitoring the pelagic system in neritic zones, i.e. in the Balearic Sea (Fernandez de Puelles *et al.*, 2003), Ligurian Sea (Licandro *et al.*, 2006), Tyrrhenian Sea (Ribera d'Alcalà *et al.*, 2004), the Adriatic Sea (Kamburska and Fonda-Umani, 2006; Bernardi Aubry *et al.*, 2012) and in the Gulf of Naples (Mazzocchi *et al.*, 2011). However, the data collected resulted to be quite heterogeneous. This could be due not only to the fact that the study covers different eco-domains with wide range of different habitats, but also to the choice of the LTER sites that was based on different motivation and thus, the measurements taken, are heterogeneous too. To define the GES indicators related to the zooplankton descriptor, representative for a certain phenomenon or sensitive to distinct changes, such as climatic-oceanographic and anthropogenic changes, and to establish and implement the future monitoring programs, an harmonization of strategies and coordinated methods along different AAs are recommended.

Bacteria

Within the MSFD, the bacterial component which represents the lower level of the trophic web, is still a matter of scientific debate. Indeed, the sole reference to bacteria in the MSFD is in terms of microbial pathogens, whose introduction is responsible for biological disturbance in the marine environment.

In the current reporting sheet on the Microbial pathogens, a methodology based on data existing under other UE Directives and National laws has been used. However, only data on faecal contamination has been taken into account, considering only intestinal enterococci and *Escherichia coli*, both recognised as the main indicators of sewage pollution. A description of the main pathogens, their spatial distribution, variation in intensity and temporal trends, are used to assess the level of pressure. The impact analysis has not been completed, since data are not yet available. It will be carried out with a description of the impacts of microbial pathogens on the marine environment (e.g mortality of biota, shifts in community structure) and main habitats and functional groups affected. Finally, in the analysis of activities, a priority list and a description of main activities according to their contribution to pressures is given.

In a future MSFD revision, there may be the opportunity of incorporating criteria and indicators which would encompass also the microbial component. Besides the commonly used indicators (enterococci, *Escherichia coli* and *Salmonella* spp.), other microorganisms such as *Vibrio* spp., enteric viruses as well as protozoa, which are recognised as emerging pathogens, are highly recommended to be included in the implementation of MSFD. Specific reference methods for their detection, which are still lacking, should also be reported.

The current version of the MSFD does not take into consideration the ecological significance of microbially-mediated processes in water biogeochemistry, in spite of their high abundance (about 10^8-10^9 cells/litre in the Mediterranean waters) and biomass of microbes (CIESM, 2000; Cotner and Biddanda, 2002; Pomeroy *et al.*, 2007). The role of bacteria in ecosystem functioning - both as decomposizers and producers - and their ability to modulate their metabolism in response to environmental changes should be taken into account in biodiversity and ecological quality monitoring programmes, as previously stated by Caruso *et al.* (2010) and Cochrane *et al.* (2010). There is an urgent need to investigate issues related to the microbial role in ecosystem functioning across a range of ecological zones, the potential influence of climate-induced warming on microbial function in marine ecosystems and the relationships between pressures and microbial function, particularly for sea-floor impacts, such as physical disturbance and organic loading. All these aspects constitute a serious knowledge gap of the MSFD and need to be addressed in its future implementation.

Conclusions - Although there is a clear recognition that planktonic communities are relevant indicators for the definition of GES in the MSFD, future research and monitoring studies have to focus on the acquisition of further data and the identification of the most useful metrics to be used at the subregional and, possibly, at the regional scale. Furthermore, even if common methodologies (Socal *et al*., 2010), and taxonomic guides (Avancini *et al*., 2006a,b) are available at the national level, training courses on plankton taxonomy and intercalibration exercises between different working groups (e.g. www.bequalm.org/phytoplankton.htm) are urgent for the implementation of the monitoring programmes.

Specifically, for phytoplankton, coordination and coherence of methods and approaches are of primary importance, as well as the strengthening of taxonomic skills, at the national and Mediterranean level. With respect to the zooplankton, support and maintenance of the Long Term Ecological Research appears to be essential to understand zooplankton dynamics: a major activity is required to deepen the knowledge of the community response and to set up an index that would combine the different metrics. Concerning the bacteria, while some monitoring activities - like those related to faecal pollution - are well stated in the current regulations, a further effort is required in order to consider, in a future implementation of the MSFD, a new integrated approach combining the study of microbial activities with that of trophodynamics. This could provide useful insights on the functional role of bacteria in organic matter turnover and nutrient recycling as well as on the susceptibility of the marine environment to pressures such as global warming and ocean acidification.

More data are needed to establish how human pressures affecting one planktonic component affect other components of the ecosystem, and to establish if there are indicators which are able to meet the majority of criteria for good indicators in a holistic ecosystem-based assessment (Painting *et al*., 2013).

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