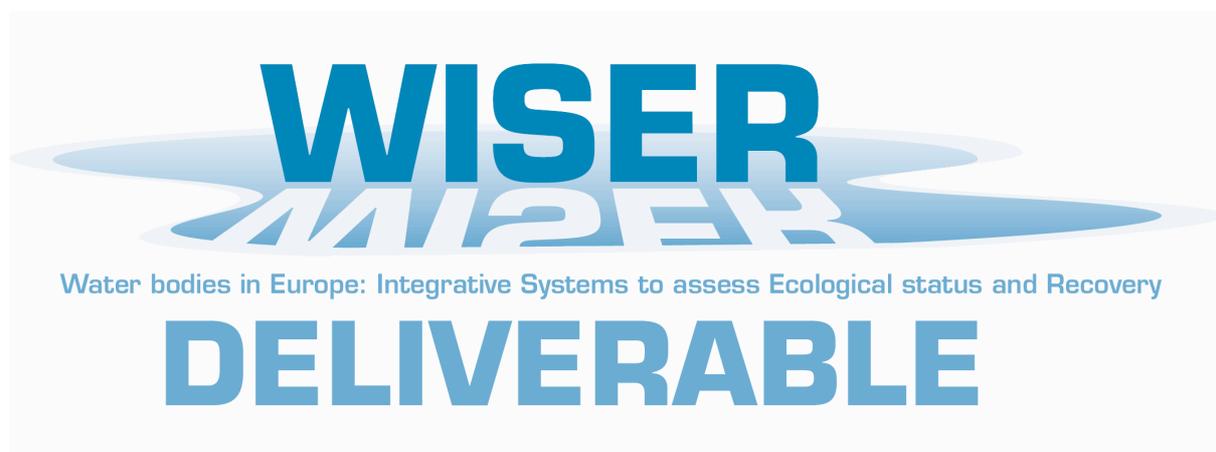


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Non-technical summary

The European Water Framework Directive (WFD) requires the Member States to assess the ecological status of the marine coastal and estuarine waters. In this assessment, several aspects of the phytoplankton communities, such as composition, abundance and biomass, must be included.

Multimetric indicators consider multiple impacts and combine individual metrics into a unitless measure to assess the overall conditions of the environment. Multimetric indicators should reduce uncertainty and increase robustness of assessment in comparison of single indicators.

In this paper, we give an overview of existing multi-species phytoplankton indicators in coastal and transitional waters, and include the phytoplankton metrics and the multimetric index (ISS-phyto) developed in coastal and transitional waters within WISER project.

1. Introduction

Much of the current environmental protection legislation requires ecosystem approach where the targets for the ecological status or health of the environment are based on the assessment of the current and desired status of the ecosystem. More specifically environmental assessment based on bioindicators that represent different aspects of ecosystem functioning and structure are requested by the European Union (EU) directives (Water Framework Directive, WFD (2000/60/EC); and Marine Strategy Framework Directive, MSFD (2008/56/EC) and also in the regional assessments carried out by the international marine conventions (e.g. HELCOM and OSPAR). The WFD require assessment of eutrophication status and impacts of other human induced pressures in surface waters, and transitional as well as in the 1 nm-wide coastal areas. The determination of the ecological quality of the water bodies is based on the biological quality elements such as phytoplankton, zoobenthos, and macrophytes, and requires monitoring and analysis of their abundance, biomass, and species composition. The MSFD includes both coastal and open sea areas (stretching to the Economic Exclusive Zone of the EU Member States), and is focused on overall health of the marine environment.

The assessment of the ecological status requires development of bioindicators that are sensitive to human pressures and can be used to set target values in minimally impacted condition or in natural baseline status, as well as to assess the deviation of the current status from the baseline. Generally indicators for the ecological assessment should meet the following criteria (Dale and Beyeler 2001): (1) They should be easily measured, (2) be sensitive to stresses on the system, (3) respond to stress in a predictable manner, (4) be anticipatory, (5) predict changes that can be averted by management actions, (6) be integrate, (7) have a known response to disturbance and changes over time, and (8) have low variability in response. According to Dale and Beyeler (2001), the suite of indicators should ideally represent key information about structure, function and composition of ecological system.

In recent years, the need of integrative tools and methods to assess ecological integrity has been recognized (Borja et al. 2008). Multi-species or multimetric indicators integrate various biological elements or individual metrics among a specific biological element. Multimetric indicators consider multiple impacts and combine individual metrics into a unitless measure to assess a site's overall conditions. Hering et al. (2006) have outlined that development of multimetric indicators should be supported since they reduce uncertainty and increase robustness of assessment in comparison of single indicators. They suggested a standardized procedure for how to develop multimetric indices comprising the following steps: (1) selection of the most suitable form of a multimetric index, (2) metric selection including exclusion of

numerically unsuitable metrics, definition of a stressor gradient, correlation of stressor gradients and metrics, definition of upper and lower anchors and scaling, (3) generation of a multimetric index, (4) setting class boundaries, and (5) interpretation of results.

The EU project WISER (Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery), aimed to develop sensitive indicators and assessment systems for those water categories and regions which are still lacking complete Biological Quality Elements (BQE) required by the WFD. In this review, we focus on the phytoplankton quality element and more specifically to review the status of the development of multi-species phytoplankton indicators in coastal and transitional waters. During the WISER project, the potential of pigment data was evaluated for multi-species and assemblage indices (Henriksen et al. 2011), and a multi-metric index of size-spectra sensitivity (ISS-phyto) was developed (Lugoli et al. 2012). A key step in the development of indicators is to describe the composition of type-specific phytoplankton communities representing high ecological quality status, i.e. near reference conditions. Within the WISER project, type-specific phytoplankton communities were identified for three ecoregions of coastal and transitional waters, and information about different methodologies for phytoplankton community studies were provided (Revilla et al. 2010a). Also the sources of uncertainty in assessment of phytoplankton communities were studied (Dromph et al. in prep.). The aim of this study was to review existing phytoplankton multimetric indices synthesized with the phytoplankton metrics and the multimetric index (ISS-phyto) developed in coastal and transitional waters within WISER project.

2. Selection of individual phytoplankton metrics

Multimetric indices can be designed differently depending on the ecosystem, purpose, organism group, and available data. Hering et al. (2006) represented the general approach and the stressor-specific approach to generate multimetric indices. In the general approach, results of individual metric values and the respective values under reference conditions are compared to derive a score for each metric. These scores are finally combined into a multimetric index. In the stressor-specific approach, metrics are selected according to their ability to detect the effects of certain stressor or the target biota. The scores of the metrics addressing a stressor are first combined into a value reflecting the intensity of the specific stressor, and the assessment results for all stressors are finally combined into a multimetric index.

Hering et al. (2006) distinguished the following metric types which can be used to form multimetric indices: (1) abundance / composition metrics (e.g. Padisák et al. 2006, Ptacnik et al.

2009, Carmendia et al. 2010), (2) richness / diversity metrics (e.g. Sherrard et al. 2006, Weckström et al. 2007, Tsirtsis and Spatharis 2009), (3) sensitivity / tolerance metrics (e.g. Lugoli et al. 2012), and (4) functional metrics (e.g. Weckström et al. 2007, Weglenska 2009, Henriksen et al. 2011). In addition to the above metrics, biomass (total biomass and the biomass of single species or groups) and frequency (e.g. blooms, Fleming and Kaitala (2006)) metrics could be included in the list to take into account the requirements of the WFD. Within the WISER project, the potential of pigment data was evaluated for multi-species and assemblage indices, but reference conditions for pigment composition could not be established due to major influence of salinity and temperature (Henriksen et al. 2011). Multimetric indices are suggested to contain about three metrics per metric type (Karr and Chu 1999, Hering et al. 2006).

Dale and Beyeler (2001) concluded that selecting only one or a few indicators often leads to poorly informed management decisions since the complexities of the ecosystem are not recognized. Thus, the suite of indicators should represent key information about (1) composition, (2) function, and (3) structure of the ecological system. When integrating a multi-species phytoplankton indicator, key characteristics of composition could include e.g. presence/absence, abundance, biomass, and frequency. Functional key characteristics could consist of e.g. population change, adaptation, growth rates, and productivity. Key characteristics in phytoplankton structure could include e.g. population structure, morphological variability, and dispersion.

3. Multimetric indices

3.1 Integration of various phytoplankton metrics

Some of the operational phytoplankton metrics are used in assessment even though integration is lacking or it is not properly described or these metrics are demonstrated only for comparison. For example, Devlin et al. (2007, 2009) have described how to use phytoplankton biomass measured as chlorophyll *a*, the frequency of elevated phytoplankton counts measuring individual species and total cell counts, and seasonal progression of phytoplankton functional groups through the year. Salmaso et al. (2006), Cheshmedjiev et al. (2010), and Pasztaleniec and Poniewozik (2010) give examples of integrating various phytoplankton metrics in lakes.

Examples also exist on phytoplankton multimetric indices which include descriptions of integration of single metrics (Table 1, 2). Revilla et al. (2009, 2010b) have developed a new

method for phytoplankton quality assessment indicating ecological quality in the coastal waters of the Basque Country (Bay of Biscay, northern Spain). This method uses chlorophyll *a* and bloom frequency. Chlorophyll *a* and bloom frequency have been combined to define also the EQS for Portuguese Transitional Waters (Brito et al. 2011). Spatharis and Tsirtsis (2010) have represented an Integrated Phytoplankton Index (IPI) which is based on chlorophyll *a*, abundance, and diversity. Lacouture et al. (2006) developed a season and salinity specific Phytoplankton Index of Biotic Integrity (P-IBI) to assess phytoplankton community status relative to estuarine nutrient and light conditions. Tett et al. (2008) have introduced Phytoplankton Community Index (PCI) measuring change, based on abundance of “life-forms” based on taxonomy, biogeochemistry, response to physical environment, and susceptibility to grazing. The data showing seasonal variation in these abundances are plotted in “life-form space” of two or more dimensions, and data from a “type-specific reference condition” are then enclosed within a reference envelope.

Moncheva and Boichenko (2011) developed an Integrated Biological Index- Phytoplankton (IBI-Ph) combining a designed combination between the following components: Integrated abundance of (Microflagelates + Euglenophytes + Cyanophytes) as a % of the total phytoplankton community abundance (MEC-%), Biodiversity Index Menhinick (1964) and Evenness Index Sheldon (1969) (Spatharis and Tsirtsis (2010), Total Abundance, and Total Biomass based on the concept of Lacouture et al. (2006), and developed a classification system for the common Bulgaria/Romania water body type for summer, validated against an Integrated Pressure Index (Moncheva and Boichenko 2011) (Table 3, Fig. 1).

Integrated multimetric phytoplankton indices for fresh waters have been developed by e.g. Salmaso (1996, 2003), Mischke et al. (2008), and Walsh and Wepener (2009).

Within WISER project, a multi-metric Index of Size-spectra Sensitivity of Phytoplankton (ISS-phyto) was developed (Lugoli et al. 2012). ISS-phyto integrates simple size spectra metrics, size class sensitivity to anthropogenic disturbance, phytoplankton biomass (chlorophyll *a*), and taxonomic richness thresholds.

Table 1. Background information on multimetric phytoplankton indices designed for coastal and transitional waters.

Name of the multimetric index	Authors and publication year	Coastal marine areas where developed and tested	Geographical areas where in operation	Legislations / Conventions
A new method for phytoplankton quality (surface-weighted EQRs)	Revilla et al. 2009, 2010	Estuaries of Basque coast	Estuaries of Basque coast	WFD
Integrated Phytoplankton Index (IPI)	Spartharis & Tsirtsis 2010	Eastern Mediterranean	Black Sea (Varna Bay)	WFD
Phytoplankton Index of Biotic Integrity (P-IBI)	Lacouture et al. 2006	Chesapeake Bay	Chesapeake Bay	
Phytoplankton Community Index (PCI)	Tett et al. 2008	United Kingdom coastal waters	United Kingdom coastal waters	MSFD, WFD
UNTRIX	Pettine et al. 2007	Italian coast	Black Sea (Varna Bay)	WFD
E.I. index for assessing eutrophication	Primpas et al. 2010	Eastern Mediterranean		WFD
Index of Size-spectra Sensitivity of Phytoplankton (ISS-phyto)	Lugoli et al. Ecological Indicators (2012, accepted with revision) Vadrucci et al. Ecological Indicators (submitted)	Black Sea (Varna Bay), northern Baltic Sea, Italian coast Lagoons in Italy, Albania, Greece, Bulgaria, Romania		WFD

Table 2. Tests and validations of multimetric phytoplankton indices designed for coastal and transitional waters. Chlorophyll a = chl a.

Name of the multimetric index	Which metrics are integrated	Relationships of individual metrics and the pressure tested	Core metrics normalized via transformation to unitless scores	Relationships of multimetric index and the pressure tested	Reference conditions established	Class boundaries established
A new method for phytoplankton quality (surface – weighted EQRs)	chl a, bloom frequency (as single taxa counts)	Yes	Yes	Yes	Yes	Yes
Integrated Phytoplankton Index (IPI)	chl a, abundance, diversity	Yes	Yes	Yes	No	Yes
Phytoplankton Index of Biotic Integrity (P-IBI)	total and taxa biomass, taxonomic composition, size structure, indicator species abundance, photosensitivity, physiological status, dissolved inorganic carbon	Yes?	Yes / No	Yes	No	No
Phytoplankton Community Index (PCI)	abundance of “life-forms” plotted in “life-form” space. “Life-forms” are based on taxonomy, biogeochemistry, response to physical environment, and susceptibility to grazing	No	Yes	No	Yes	No
UNTRIX	chl a, oxygen deficit, dissolved inorganic nitrogen, total phosphorus	Yes	Yes	Yes	Yes	No
E.I. index for assessing eutrophication	chl a, nitrate, nitrite, ammonia, phosphate	No	Yes	Yes	No	Yes
Index of Size-spectra Sensitivity of Phytoplankton (ISS-phyto)	size spectra metrics, size class sensitivity to anthropogenic disturbance, phytoplankton biomass (chlorophyll a), and taxonomic richness thresholds	Yes	Yes	Yes	No	Yes

Table 3. Pressure scores by activities and sites within the common Bulgaria-Romania- water body types. (score: 0- no impact, 1-low, 2-medium, 3-strong).

Station Name	Agricultural diffuse inputs	River discharge	Domestic discharges/WWTP (nutrients)	Industrial discharges	Organic loads (BOD5)	Urbanization	Tourism (nutrients)	Port activity	TOTAL PRESSURES	Pressure Index	EQR-IBI	IBI
Cazino	3	2	1	1	1	1	1	1	11	1.38	0.84	3.36
Constanta	3	2	1	3	3	2	3	3	20	2.50	0.59	2.34
Eforie	3	1.5	0.5	1.5	1.5	0.5	1	1.5	11	1.38	0.74	2.97
Costinesti	3	1.5	0	1	1	0.5	1	1.5	9.5	1.19	0.80	3.19
Mangalia	3	1	0	2	2	1	2	2	13	1.63	0.79	3.15
Vama Veche	3	1	0	1	1	0.5	0.5	0.5	7.5	0.94	0.81	3.25
Krapetz	2	1	2	0	2	1	1	0	9	1.13	0.69	2.74
Balchick	1	0	1	1	1	1	3	1	9	1.13	0.80	3.21
VG	0	0	1	1	1	1.5	1.5	2	8	1.00	0.71	2.84
VB-III	0	0	3	2	3	3	3	1	15	1.88	0.62	2.47
VB	2	0	3	3	3	3	3	3	20	2.50	0.53	2.12
Kamchia	3	3	1	0	2	1	1	0	11	1.38	0.73	2.93

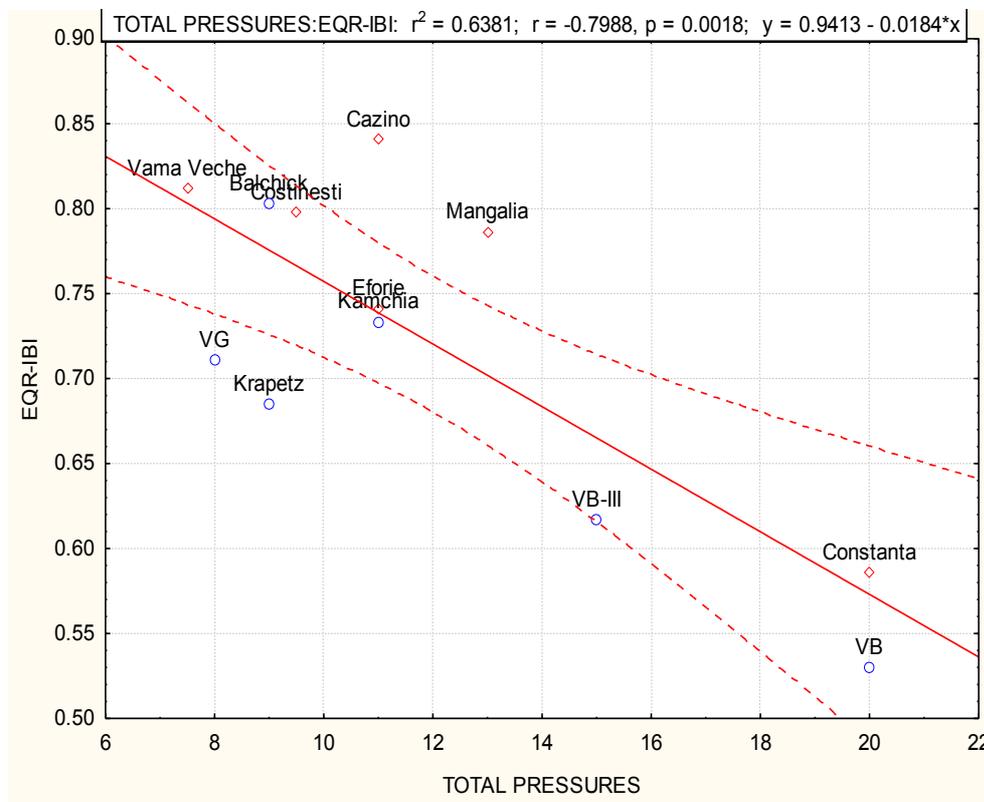


Fig.1. Scatter plot of Integrated Biological Index (IBI-EQR) to the Total pressure score by stations (integrated BG and RO).

3.2 Phytoplankton metrics integrated with metrics of other biological or water quality elements

Multiple quality elements and statistical multivariable tools are in use when assessing overall ecological status of marine and fresh water ecosystems (Salmaso and Padisák 2007, Giordani et al. 2009, Rask et al. 2011). In the combination of the different quality elements into an overall assessment, the various methods to combine the parameter values become important in determination of the final assessment. Multimetric indices may constitute of both biological and water quality elements together. The uncertainty related the different combination approaches and methods is dealt in the work package 6.2 of the WISER project.

For instance, multimetric methods can be used to combine several quality elements into a single value of an indicator or as in the WFD, into a unitless Ecological Quality Ratio. Also averaging of the different parameters and one-out-all-out (basing the final assessment on the value of the lowest of the parameters or quality elements) are approaches being applied. For example, this approach has been used in the eutrophication and biodiversity assessment of HELCOM, using their HEAT and BEAT tools (Andersen et al. 2010). Also, Kane et al. (2009) have integrated phytoplankton and zooplankton metrics into a multimetric index applied in lake assessments.

The composite trophic status index (TRIX) is based on an absolute trophic scale without normalization to type-specific reference conditions, and it makes an aggregation of biological (chlorophyll *a*) and physico-chemical (oxygen, nutrients) quality elements. Based on the TRIX methodology, Pettine et al. (2007) presented an unscaled TRIX (UNTRIX) which fulfils the requirements of the WFD. The UNTRIX is calculated using the log-transformation of the product of four eutrophication-related variables (chlorophyll *a*, oxygen deficit, dissolved inorganic nitrogen and total phosphorus). By using principal component analysis, Primpas et al. (2010) developed an eutrophication index which is a linear combination of chlorophyll *a*, nitrate, nitrite, ammonia, and phosphate with almost equal weights.

According to the WFD, ecological status is assessed based on ecological quality ratios (EQR), describing the relationship between reference conditions and the present value of a metric. Sagert et al. (2008) and Alahuhta et al. (2009) demonstrated how to integrate EQRs as a kind of multimetric index instead of using the "one-out, all-out" principle required by the WFD in assessing ecological status based on each biological quality element. Sagert et al. (2008) used a normalization procedure in integration, whereas Alahuhta et al. (2009) harmonized the individual measures by scoring and expressed then the overall status class as a median score across the quality elements.

4. Conclusions

During recent years, the importance of multivariate indicators in increasing robustness and reducing uncertainty of assessment of the health of marine environment has been recognized (Hering et al. 2006, Borja et al. 2008). In this study, we reviewed existing phytoplankton multimetric indices in coastal and transitional waters. We also took into account the phytoplankton metrics and the multimetric index (ISS-phyto) developed within the WISER project.

Many of the multimetric indices lack the establishment of reference conditions and WFD class boundaries, which makes it impossible to use them directly for WFD assessment. These indices may still be very informative on the health of the environment, and may fulfil the requirements of other legislations or directives, such as the MSFD. Phytoplankton monitoring is needed not only to indicate changes in eutrophication levels, but also to monitor the changes in the presence of toxic and harmful species, invasive species, and biodiversity.

Harmonized lists of phytoplankton taxa, counting formulas for biovolumes, sampling and analyzing methods, and well-educated microscopists form the basis for reliable phytoplankton monitoring results. Thus, European-wide methodology guides, taxa lists, formulas for biovolume calculations, and intercalibration tests for microscopists are needed, and should be encouraged to increase the reliability and further usefulness of the phytoplankton monitoring data.

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