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Methods of eutrophication assessment in the context of the water framework directive: Examples from the Eastern Mediterranean coastal areas

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ABSTRACT

A set of methodological tools were tested in order to assess the eutrophication quality of selected coastal areas in Eastern Mediterranean Sea, Greece, in the context of the Water Framework Directive under various anthropogenic pressures. Three, five-step tools, namely, TRIX, chlorophyll-a (chl-a) biomass classification scheme, and eutrophication index (E.I.) were applied in oligotrophic waters for (a) the whole water column and (b) the euphotic zone. The relationship among the eutrophication assessment indices and the ecological quality status (EcoQ) assessment indices for benthic macroinvertebrates (BENTIX index) and macroalgae (ecological evaluation index-EEIc) was also explored. Agricultural activities and mariculture are the pressures mostly related to the eutrophication assessment of the selected Greek coastal water bodies. Chl-a proved to be the criterion with the best overall correlation with the EcoQ indices, while TRIX with the lowest. Moreover, among the eutrophication indices, E.I. showed better overall agreement with BENTIX showing that probably it reflects the indirect relation of macroinvertebrates with water eutrophication in a better way. Among the eutrophication indices used, TRIX rather overestimated the eutrophication status of the selected coastal areas. The first stage of eutrophication was reflected more efficiently using E.I. than TRIX, but E.I. seems to be a rather sensitive index. A future modification of the high to good boundary of E.I. may be needed in order to demonstrate the high status of the relatively undisturbed Greek coastal sites.

1. Introduction

Coastal marine environments are usually influenced by human-induced and natural pressures, which may alter their functioning, and finally contribute to ecosystem degradation and pollution problems (Jickells, 2005; Aubry and Elliott, 2006; Borja et al., 2010). The legislation developed and applied worldwide includes restoration of degraded aquatic habitats as one of the primary goals and require suitable methods to assess their quality in relation to anthropogenic impacts on marine ecosystems using various elements of the ecosystem (Borja et al., 2008, 2011, 2012; Ferreira et al., 2011). In Europe, the umbrella regulations for addressing the ecological quality of the water systems are the Water Framework Directive (WFD; 2000/60/EC), for lakes, rivers, transitional (estuaries and lagoons) and coastal waters, and the Marine Strategy Framework Directive (MSFD; 2008/56/EC) for all marine waters (Van Hoey et al., 2010). An informative work by Borja et al., (2010) presents a system of applying the experience gained from the WFD to implement the MSFD. This work outlines the points of overlap and conflict between the two directives and is regarding the WFD as a 'deconstructing structural approach' whereas the MSFD is a 'holistic functional approach', i.e. the WFD has split the ecosystem into several biological quality elements (BQEs) and evaluates them individually before combining them and attempting to determine the overall condition. The other elements (hydromorphological and physicochemical) are only used to support the BQEs.

In contrast the MSFD focuses on the set of 11 descriptors with several indicators covering the ecological, physical, chemical and anthropogenic components of the ecosystem that need to be integrated at the indicator and descriptor levels (Van Hoey et al., 2010). These 11 descriptors together summarize the way in which

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the whole system functions. Moreover, MSFD has established a framework for the development of strategies designed to achieve good environmental status (GEnS), which takes into account the structure, function, and processes of the marine ecosystems together with natural physiographic, geographic, and climatic factors, as well as physicochemical conditions including those resulting from human activities in the area concerned (Borja and Collins, 2009).

The ecological quality status (EcoQ) within the WFD and the environmental status (ES) within the MSFD should be harmonized and the two directives should be fully and seamlessly integrated (Borja et al., 2010). To this respect, intercalibrated indices which are used under the WFD can also assess the ES within the MSFD in the respective interlinked criteria or indicators. Such an approach addressing interlinking quality elements' indicators and descriptors of both directives has been applied in several cases i.e. the Basque country (Borja et al., 2011) and Greece (Simboura et al., 2015). The intercalibrated and interlinked indicators used in the present work are the chlorophyll-a (chl-a) biomass (pertaining to eutrophication descriptor 5) and the benthic and macroalgae indices (pertaining to biodiversity and sea floor integrity descriptors, namely, D1 and D6, respectively).

Regarding the eutrophication, the WFD intends to improve the ecological status, including eutrophication status, of all European surface waters of which many are considered to be eutrophic (European Environment Agency, 2001, 2003). However, according to Andersen et al. (2006), the WFD does not explicitly consider eutrophication because the treatment of eutrophication is indirect with the boundary between good and moderate ecological status being defined as an environmental management objective. Consequently, the need for a common understanding and definition of eutrophication, as well as, the need for stronger coordination between directives dealing directly or indirectly with eutrophication has been emerged (Andersen et al., 2006; Ferreira et al., 2011). It is important to point out that the WFD is a dynamic directive and permits further incorporation of new methodologies, or improvements of those already applied (Revilla et al., 2009). On the other hand, MSFD takes a functional approach to eutrophication establishing it as one of the 11 holistic quality descriptors, namely, descriptor 5 (MSFD; 2008/56/EC; Ferreira et al., 2011). This is important because eutrophication problems have been reported from a wide variety of coastal ecosystems (Justic et al., 2005; O' Higgins and Gilbert, 2014).

The guidance for the descriptor 5 (D5) defines that most eutrophication assessment methods recognize that the immediate biological response is increased primary production reflected as chl-a and/or macroalgal abundance (Ferreira et al., 2010). These are "direct effects" or "primary symptoms" and indicate the first stages of eutrophication. "Indirect effects" or "secondary symptoms" such as low dissolved oxygen (DO), losses of submerged aquatic vegetation (SAV), changes in macrozoobenthic species composition, and occurrences of nuisance and toxic blooms indicate more advanced problems.

Various methods have been developed in the EU to assess eutrophication in order to fulfill requirements of legislation designed to monitor and protect coastal water bodies from degradation. Some methods use only chl-a concentrations, while in others chl-a concentrations are combined with other parameters to give a more integral picture of eutrophication (Borja et al., 2012). However, in many cases the various methods give different assessment results in terms of classes when they are applied to the same water body. In such cases, we have to decide which method is more efficient and representative of the condition in determining the eutrophication status (Borja et al., 2012).

The Eastern Mediterranean Sea has always been considered as one of the most oligotrophic areas in the world with extremely low nutrient concentrations, 12 times lower than the Atlantic Ocean (Pavlidou and Souvermezoglou, 2006; Krom et al., 2010). Despite the oligotrophic character of the Mediterranean Sea, elevated nutrient concentration indicates coastal eutrophication problems because several coastal areas undergo intense and continuous environmental pressure derived from a number of driving forces such as urbanization, industrialization, changes in land use, tourism development, aquaculture development, climate change, and others (Pavlidou and Souvermezoglou, 2006; UNEP, 2007; Halpern et al., 2007; Karydis and Kitsiou, 2013; Pascual-Aguilar et al., 2015; Levin et al., 2014; Newton et al., 2014). In Greece, more than 80% of the industrial activities and 90% of tourism activities are located along the coastline (Anagnostou et al., 2005). Athens and Thessaloniki, the two biggest cities of Greece, exceeding 4 and 1 million people, respectively, are also located on the coastal zone and influence the functioning of Saronikos and Thermaikos ecosystems, in the central and northwestern Aegean Sea, respectively (Anagnostou et al., 2005; Karageorgis et al., 2005; Konstantinou et al., 2012; Pavlidou et al., 2014).

This study was conducted in selected coastal areas of Greece, influenced by the human activities and which are subjected to different types of pressure. The eutrophication status of these areas was studied using different indicators and different methodological approaches in the context of WFD. The WFD tries to combine both aspects of pressures and biological elements (water and benthic) into a sole ecological status. In these terms, results of the eutrophication status of the coastal water bodies were juxtaposed and compared to the benthic indices results.

The objectives of this work are (i) to identify, evaluate, and map the different types of pressures affecting each area; (ii) to assess the eutrophication status of the studied coastal areas based on three different assessment principles and methods usually applied in Greek ecosystems; (iii) to compare the resulting classifications and evaluate them; (iv) to compare the eutrophication status with the benthic status of the coastal areas and investigate whether there is a good link between them or not.

2. Materials and methods

2.1. Monitoring program

WFD requires that EU member states must regularly monitor and report on the condition of the coastal water bodies within their jurisdiction (Ferreira et al., 2007). However, reviewing the objectives and requirements of marine water quality monitoring, Karydis and Kitsiou (2013) highlighted the scarcity of the marine monitoring programs.

A national monitoring program for coastal waters is undertaken and run by the Hellenic Centre for Marine Research (HCMR) in Greece (Simboura et al., 2015). The monitoring network has been designed for the implementation of WFD in coastal waters and is delegated by the Greek water management authorities. The Greek authorities report annually on the water quality status to the European Environment Agency providing data sets of physical characteristics and concentrations of inorganic and organic nutrient, organic matter, chl-a, macroalgae and macroinvertebrates and hazardous substances together with the characterization of the main pressures and impacts from human and other activities at each monitoring station, according to Annex V of WFD 2000/60/EC (Anonymous, 2012).

For this study, we have used data from 27 coastal monitoring stations located in 15 water bodies of Greece which are subjected to different types of anthropogenic pressures. Among them, the station in Limnos Island in the Aegean Sea receives very minor anthropogenic pressures (Fig. 1; Table 1; see Section 2.2). In this

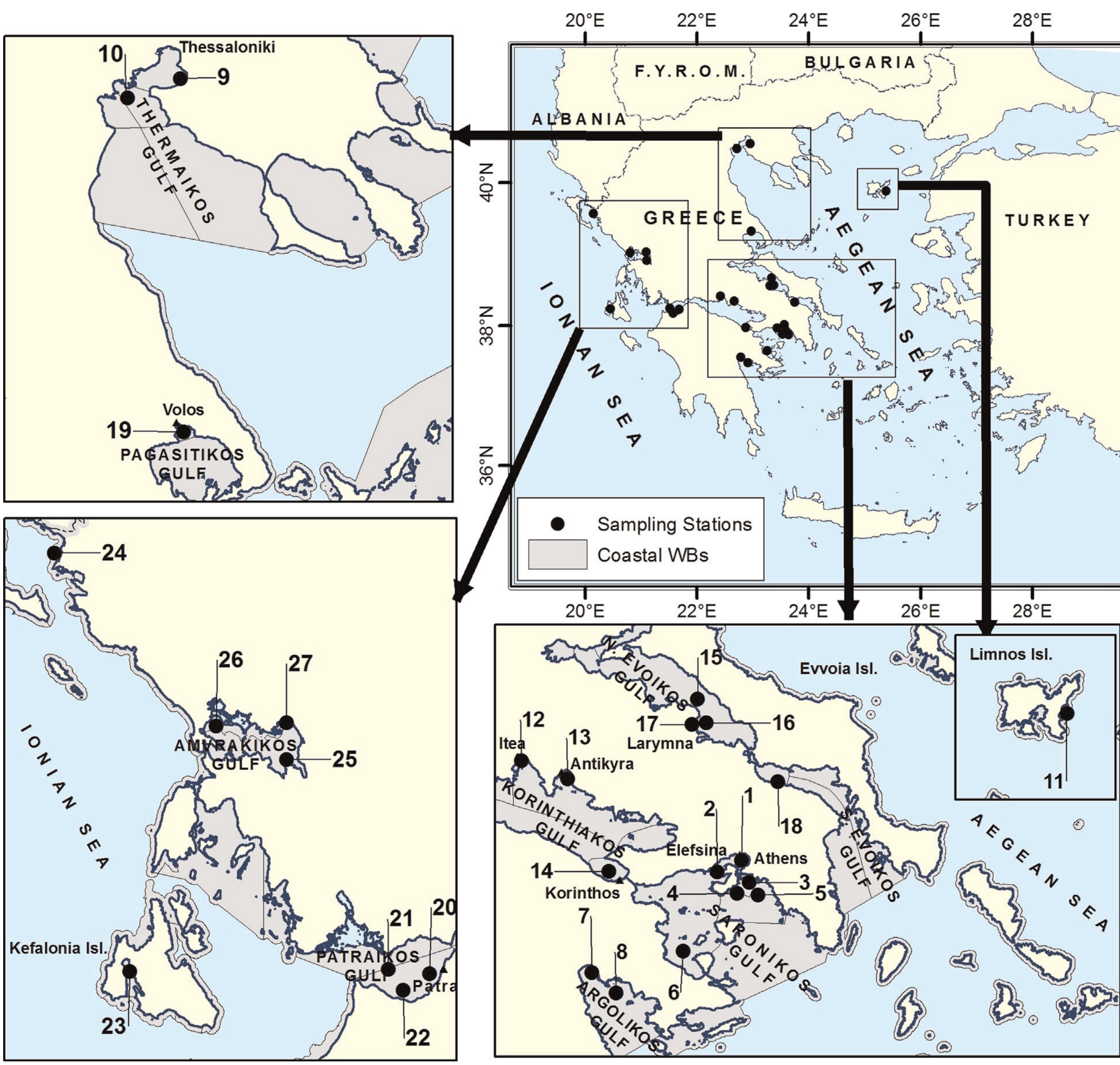

Fig. 1. Monitoring stations and water bodies.

work, we used data from five sampling periods during 2012–2014 (March–April 2012; November 2012; March–April 2013; November 2013; March–April 2014).

2.2. Sites description and pressures

The 15 water bodies of Greece where this study is focused, are Elefsis bay, Inner Saronikos gulf, Western Saronikos gulf, Korinthiakos gulf, Patraikos gulf, Amvrakikos gulf, Argolikos gulf, N. Evoikos gulf, S. Evoikos Gulf, Pagasitikos gulf, Thermaikos gulf, Thessaloniki bay, Argostoli bay, Coasts of Ionian Sea, and Aegean Sea (Fig. 1; Table 1). The coastal areas are impacted by several human activities such as treated and untreated sewage and industrial discharges, agricultural/livestock farm discharges, aquaculture (in general, finfish but in some cases e.g. Thermaikos gulf and Maliakos gulf, shelfish production also takes place), urbanization and tourism, and so on, whereas the Aegean Sea (Limnos Island) has been considered as less impacted by any of the aforementioned anthropogenic activities area (Anagnostou et al., 2005; UNEP, 2007; Pavlidou, 2012). Many of these pressures have been recognized to be related to eutrophication status (Ferreira et al., 2011). Table 2 summarizes the characteristics of the study areas and anthropogenic pressures that affect them.

In this work, we have tried to assess the distribution of anthropogenic pressures on the coastal water bodies of Greece. A pressure index has been estimated (Aubry and Elliott, 2006; Borja et al., 2010, 2011) for the different coastal areas to give the magnitude of the anthropogenic pressures imposed. The pressure intensity scale of Borja et al., (2011) has been modified to include five levels of evaluation and assigning scores from 3 to 0 for each pressure type within the corresponding area. The scores were estimated using expert judgment based on our knowledge of the study areas. The pressure index has been calculated as the average pressure scores and the selected areas have been grouped in five categories from no or minor pressure intensity to heavy pressure intensity (Table S1). The pressure types include sewage discharge, industrial discharge, agricultural discharge, spoil waste, mariculture, fishing, marinas, and ports based on the Water Information System for Europe (WISE-SoE), a reporting European system for coastal and marine waters (http://cdr.eionet.europa.eu/gr/eea). Lastly, a map has been produced showing the pressure status and composition on a station basis.

Table 1
Coastal monitoring stations used for analysis.

Station ID	Monitoring Stations				Coastal Water Body		Water District	Stations' Characteristics	
	Name	Name	Coordinates (wgs84)		Name	Code	Name	Max Depth (m)	Average Secchi Disk Depth (m)
			lon	lat					
1	S1	Elefsina Bay	23.55750	38.01810	Elefsis Bay	GR000600010007H	Attica	22	7
2	Faneromeni	Faneromeni Bay	23.43110	37.97126	Elefsis Bay	GR000600010008N	Attica	20	7
3	S7	Inner Central Saronikos - Psitalleia	23.59580	37.92830	Inner Saronikos Gulf	GR000600010006H	Attica	70	12
4	S8	Inner (Central) Saronikos	23.53330	37.88330	Inner Saronikos Gulf	GR000600010005N	Attica	90	16
5	S11	Inner (Central) Saronikos	23.64170	37.87670	Inner Saronikos Gulf	GR000600010005N	Attica	75	15
6	S25	Western Saronikos Gulf	23.25560	37.64720	Western Saronikos Gulf	GR000600010009N	Attica	415	20
7	Argolikos	Argolikos Gulf	22.78500	37.55970	Argolikos Gulf	GR000300010002N	Eastern Peloponnese	16	5
8	Vourlias	Argolikos Gulf	22.90930	37.47700	Argolikos Gulf	GR000300010002N	Eastern Peloponnese	180	17
9	TP10	Thessaloniki Bay	22.95140	40.53580	Thessaloniki Bay	GR001000010010H	Central Macedonia	20	4
10	TP16	Inner Thermaikos Gulf - N. Mhxaniona	22.71750	40.46920	Inner Thermaikos Gulf	GR001000010009N	Central Macedonia	28	4
11	Limnos	Limnos coasts	25.38329	39.88268	Open Aegean Sea	GR001400010002N	Aegean Islands	80	19
12	Itea	Itea Bay	22.42245	38.42384	Korinthos Gulf	GR000700010014N	Eastern Greece Hellas	20	9
13	Antikyra	Antikyra Bay	22.66047	38.35119	Korinthos Gulf	GR000700010013H	Eastern Sterea Hellas	70	18
14	Korinthos	Korinthos Bay	22.87206	37.97522	Korinthos Gulf	GR000200010006N	Northern Peloponnese	98	13
15	Theologos	N. Evoikos Gulf	23.33060	38.67310	N. Evoikos Gulf	GR000700010007N	Eastern Sterea Hellas	65	11
16	Skouries	N. Evoikos Gulf	23.37500	38.57780	N. Evoikos Gulf	GR000700010007N	Eastern Sterea Hellas	75	12
17	Larymna	Larymna Bay	23.30080	38.57190	N. Evoikos Gulf	GR000700010008H	Eastern Sterea Hellas	18	7
18	Asopos	Avlida Bay	23.74470	38.33920	S. Evoikos Gulf	GR000700010006N	Eastern Sterea Hellas	10	5
19	Volos	Volos Bay	22.96670	39.33330	Pagasitikos Gulf	GR000800010005H	Thessaly	40	10
20	Patra	Patraikos Gulf	21.67890	38.23620	Patraikos Gulf	GR000200010004N	Northern Peloponnese	100	11
21	W. Patraikos	Patraikos Gulf	21.51000	38.25000	Patraikos Gulf	GR000200010004N	Northern Peloponnese	90	9
22	S. Patraikos	Patraikos Gulf	21.57110	38.18140	Patraikos Gulf	GR000200010004N	Northern Peloponnese	60	11
23	Argostoli	Argostoli Bay	20.45170	38.24170	Argostoli Bay	GR000200010014N	Northern Peloponnese	18	7
24	Kalamas	Eastern coasts of Corfu Sea	20.14390	39.57750	Coats of Ionian Sea	GR000500010007N	Epirus	12	6
25	S.Amvrakikos	S. Amvrakikos Gulf	21.09450	38.92150	Amvrakikos Gulf	GR000500010002N	Epirus	40	2
26	Louros Estuary	Northern Amvrakikos Gulf	20.80463	39.03025	Amvrakikos Gulf	GR000500010001N	Epirus	16	3
27	Arachthos Estuary	Northern Amvrakikos Gulf	21.09420	39.03920	Amvrakikos Gulf	GR000500010001N	Epirus	20	2

Table 2
Characteristics of the sampling sites and anthropogenic pressures affecting them.

Station ID	Station name	Water body name	Pressures	Other characteristics
1	S1	Elefsis Bay	Industrial effluents (Refineries, shipyards, chemical plants, food, metal, cement industries etc)	Hypoxic events, Algal Blooms
2	Faneromeni			
3	S7	Inner Saronikos Gulf	Treated Sewage from Athens	
4	S8			
5	S11			
6	S25	Western Saronikos Gulf	Maricultures and agricultural discharges.	Hypoxic and anoxic events during the last 22 years
7	Argolikos	Argolikos Gulf	Agricultural	
8	Vourlias			
9	TP10	Thessaloniki Bay	Thessaloniki harbor, industrial, treated or partly treated sewage	Oxygen decrease
10	TP16	Inner Thermaikos Gulf	Agricultural discharges from the heavily polluted Axios River, mariculture	
11	Limnos	Open Aegean Sea	Very minor pressures	
12	Itea	Korinthos Gulf	Domestic and industrial effluents	
13	Antikyra			
14	Korinthos		Harbor activities	
15	Theologos	N. Evoikos Gulf	Agricultural, maricultures	
16	Skouries		Industrial (smelting plant discharge)	
17	Larymna			
18	Asopos	S. Evoikos Gulf	Industrial and agricultural	
19	Volos	Pagasitikos Gulf	Sewage, industrial and harbor activities	
20	Patra	Patraikos Gulf	Harbor and industrial activities	
21	W. Patraikos		Industrial and agricultural	
22	S. Patraikos			
23	Argostoli	Argostoli Bay	Aquacultures and tourism activities	
24	Kalamas	Coats of Ionian Sea	Agriculture and other activities	
25	S.Amvrakikos	Amvrakikos Gulf	Agriculture	Anoxic conditions
26	Louros Estuary			Hypoxic conditions
27	Arachthos Estuary			

2.3. Eutrophication assessment

In this study, we have assessed the eutrophication quality of the selected coastal Greek areas using three, five-step, different tools applied in the oligotrophic waters of the Eastern Mediterranean coastal areas: (i) the trophic index TRIX (Vollenweider et al., 1998; Primpas and Karydis, 2011); (ii) chl-a biomass classification scheme (Simboura et al., 2005; Pagou et al., 2002); and (iii) eutrophication index (E.I.) (Primpas et al., 2010).

TRIX was calculated according to the following equation based on Vollenweider et al (1998), whereas eutrophication ranges have been modified and applied for the oligotrophic areas of Eastern Mediterranean according to Primpas and Karydis (2011):

$$TRIX = \left[\log_{10}(C_{PO4} * C_{DIN} * C_{Chla} * D\%O_2) + 1.5\right]/1.2$$

The E.I. was calculated according to the following equation (Primpas et al., 2010):

$$E.\,I. = 0.279 * C_{PO4} + 0.261 * C_{NO3} + 0.296 * C_{NO2} + 0.275 * C_{NH4} + 0.261 * C_{Chla}$$

where

C_{DIN} is the concentration of dissolved inorganic nitrogen ($= C_{NO3} + C_{NO2} + C_{NH4}$); C_{PO4} is the concentration of phosphate; C_{NO3} is the concentration of nitrate; C_{NO2} is the concentration of nitrite; C_{NH4} is the concentration of ammonium (nutrient concentrations for TRIX in mg*m^{-3};for E.I calculation in mmol m^{-3}); C_{Chla} is the concentration of phytoplankton chl-a (in mg m^{-3}). D%O_2 is the % deviation of the oxygen concentration from saturation conditions.

Table 3 shows the different methods used for the eutrophication assessment, the indicators used for each methodological tool, the classes of eutrophication status, and the eutrophication range. Nutrient, DO and chl-a data were measured using standard methods and quality assurance protocol according to the ISO 17025 certification procedures (Mullin and Riley, 1955; Murphy and Riley, 1962; Holm-Hansen et al., 1965; Carpenter, 1965; Koroleff, 1970; Strickland and Parsons, 1977; Welschmeyer, 1994).

Table 3
Methodological tools, indicators, and ranges used for Greek coastal areas for the eutrophication assessment.

Methods	Indicators	Eutrophication status	Eutrophication Range
TRIX[a,b]	D%O_2, DIN (= NO_3^- + NO_2^- + NH_4^+), PO_4^{3-}, Chl-a	High	< 1.6
		Good	1.6–2.8
		Moderate	2.8–4.0
		Poor	4.0–5.3
		Bad	> 5.3
Chl-a biomass classification scheme[c,d]	Chl-a	High	< 0.1 (mg m^{-3})
		Good	0.1–0.4 (mg m^{-3})
		Moderate	0.4–0.6 (mg m^{-3})
		Poor	0.6–2.21 (mg m^{-3})
		Bad	> 2.21 (mg m^{-3})
E.I.[e]	NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, Chl-a	High	< 0.04
		Good	0.04–0.38
		Moderate	0.38–0.85
		Poor	0.85–1.51
		Bad	> 1.51

[a] Vollenweider et al. (1998).
[b] Primpas and Karydis (2011).
[c] Simpoura et al. (2005)
[d] Pagou et al. (2002).
[e] Primpas et al. (2010).

The coastal areas have been classified according to each methodological tool. Nutrient, DO and chl-a data from the whole water column, as well as from the layer of the euphotic zone (determined as three times the Secchi disk disappearance depth) were used. Thematic maps presenting the eutrophication assessment according to the three different methodological tools were produced (a) for the whole water column and (b) for the euphotic zone. The thematic maps were produced in ArcGIS/ArcINFO environment on station basis.

2.4. Benthic indices

Benthic ecological status indices are influenced mostly by the pressures affecting the sea bottom, while water column indices by pressures in the water column. However, the benthic macroinvertebrates are considered as good indicators of general environmental status due to their limited ability to move and avoid pressures and also due to their low temporal variability and long life cycles (Borja et al., 2009; Prins et al., 2013).

Therefore, two intercalibrated (GIG, 2013) EcoQ benthic indices were used in order to juxtapose and compare the classification according to eutrophication indices with the one resulting from these ecological quality indices. The BENTIX index (Simboura and Zenetos, 2002) was used for the classification of the EcoQ of the benthic macroinvertebrate communities, while for the ecological evaluation of the biological element of macroalgae, the EEIc (Orfanidis et al., 2001, 2003, 2011) was used.

Within the intercalibration exercise, it has been found that diversity measures of the benthic community did not show monotonic patterns of response to the gradient of organic content, while strong correlations were found between indicator taxa indices and the pressure gradient. Diversity indices have also been criticized as ecological status indicators due to their dependency on habitat type, natural variations, and taxonomic effort. In order to cover the MSFD requirements for structural components of the benthic community, a multimetric benthic formula has been developed (Simboura et al., 2015) including biotic and structural components in a formula controlling diversity components contribution. However, this formula results in a similar EcoQ classification with the biotic index at most cases, and it was not used here.

2.5. Statistical analysis

To analyze the agreement among eutrophication and ecological quality status indices, as well as among eutrophication indices, a weighted Kappa analysis was undertaken (Cohen, 1960; Landis and Kosch, 1977) applying the methodology presented in Borja et al., (2007). This analysis takes into account that the importance of misclassification is not the same among the close categories (e.g. high or good, moderate or poor) as among other categories (e.g. between high or good and moderate or poor). The Kappa values reveal the next levels of agreement: (i) null <0.05; (ii) very low: 0.05–0.2; (iii) low: 0.2–0.4; (iv) moderate: 0.4–0.55; (v) good: 0.55–0.7; (vi) very good: 0.7–0.85; (vii) almost perfect: 0.85–0.99; and (viii) perfect: 1 (Monserud and Leemans, 1992).

In addition, the SPSS software program was used to explore correlations across eutrophication results and selected indices. For Pearson correlations, the index values were standardized by using $\log_{10}$ transformation of values. Factor analysis was rotated using the Varimax rotation method and was used in order to investigate the relative importance of the human pressures on eutrophication and nutrient ratios.

3. Results and discussion

3.1. Human-induced pressures

The need to assess eutrophication in Greek coastal waters appropriately and in relation to different pressures derives from the need to clarify the responsiveness of the ecological indices/metrics elements to the different pressures within the implementation of the European Directive in Greece.

The resulting ranking of the selected areas based on the priority score (pressure index) given to each type of pressure for each station allowed us to assess the relative importance of each area compared to the others (Table S1; Fig. 2). Industrial discharges, port activities, sewage discharges, aquaculture activities, and fishing are the most important pressures affecting the coastal areas of Greece. In fact, maricultures seem to affect more the selected coastal areas among the anthropogenic pressures defined (Table S1), followed by fishing, other activities and industrial discharges. This is interesting, since mariculture activities in Greece have expanded rapidly during the last years. Moreover, the impact of mariculture activities on the water column of some Greek coastal areas has been reported, indicating relatively elevated nutrient concentrations (Pavlidou and Rousselaki, 2014).

According to Pearson analysis, chl-a, TRIX, and E.I. correlated positively with the pressure index with correlation coefficients of 0.72, 0.68, and 0.56, respectively. More specifically, in both data treatments, chl-a, E.I., and TRIX correlated significantly (at the level 0.01) with agricultural pressures and mariculture, whereas they did not correlate with sewage and industrial discharges and port activities (Tables S2 and S3).

A factor analysis (FA) for the pressures and eutrophication indices was performed for the entire water column data treatment (Figure is not presented) as well as for the euphotic zone (Fig. 3). The analysis for the entire water column resulted to three main factors or components (75% of total system variability was explained; 39% by the first factor, 27% by the second, and 9% by the third). Fishing activities, mariculture, and discharges from agricultural activities together with chl-a and other activities acquired high loadings in the first component, sewage and industrial discharges, spoil wastes, and ports in the second component, while the eutrophication indices E.I. and TRIX cluster together in the third component and acquire high loadings together with marinas (Table S4). The eutrophication indices, that is, E.I. and TRIX, seem to reflect the integral effects of the pressures exerted on the coastal water bodies of Greece.

FA for the euphotic zone resulted to two main factors (71% of total system variability was explained, 40% by the first factor, and 31% by the second). Chl-a, E.I., and TRIX together with fishing, agricultural activities, mariculture, and other activities acquired high loadings in the first component (Table S5). In the second component, sewage and industrial discharges, ports, and spoil wastes are the main pressures. It seems that, agricultural and maricultural activities, fishing, and other activities (riverine discharges, dredging, etc.) couple with all eutrophication status indices when we refer to the euphotic zone only.

From this analysis, it seems that two main types of pressures affect the selected coastal areas: the pressures related to agricultural and maricultural activities and those related to sewages, industries, and ports. Thus, we can recognize two different drivers for these pressures: nutrients related to agricultural activities, and inorganic and organic pollutants (metals and organic compounds) related to industries, sewages, and ports. The eutrophication indices which are discussed in this work seem that are mainly connected or affected by the agricultural activities, mariculture, fishing, and other activities. Thus, the eutrophication indices in the euphotic zone spontaneously express the direct effects of eutrophication driven by the nutrient enrichment.

3.2. Nutrient and nutrient ratios

Spatial variation of nutrient concentrations among the coastal stations was observed. Nitrate concentrations ranged between

Fig. 2. Assessment of the pressure level in terms of relevant pressures and water body.

0.01 and 23.5 mg m^{-3}. The highest nitrate value was recorded at station 10 in the inner Thermaikos gulf. Phosphate concentrations ranged between 0.01 and 2.25 mg m^{-3} and the highest value was recorded near the bottom of station 25 in the south Amvrakikos gulf, where anoxic conditions have been developed. Nitrite concentrations ranged between 0.01 and 1.54 mg m^{-3} (maximum concentration near the bottom of station 27 in Amvrakikos gulf, close to the mouth of Arachthos). Ammonium concentrations in the coastal areas of Greece ranged between 0.05 and 5.06 mg m^{-3} (maximum value recorded near the bottom of station 21). Chl-a concentration in the Greek coastal areas ranged between 0.01 and 6.18 mg m^{-3}. High phytoplankton biomass was recorded near the bottom (17 m) of station 26 close to Louros mouth in Amvrakikos gulf.

Concerning the different forms of dissolved inorganic nitrogen (DIN), nitrite (NO_2^-) was the predominant form only in 3% of the data. On the contrary, nitrate (NO_3^-) was the dominant form in the 58% of data, whereas ammonium (NH_4^+) was the predominant form in the 39% of the data.

The mean integrated DIN:P ratios calculated for the entire water column were much higher than the Redfield ratio (16:1) in most of the cases (82% of the stations) with average mean integrated value of all stations 37 ± 28, except in the inner Saronikos gulf (stations 3 and 5), Amvrakikos gulf (stations 25 and 26), and Thessaloniki bay (station 9), which were defined as nitrogen limited areas. DIN:Si ratio was calculated lower than 1 (average value: 0.47 ± 0.20) in the majority of the study stations indicating silicate excess in the monitoring stations. The analysis of our data showed spatial variation of the DIN:P ratio.

The atomic Si:DIN:P ratio of marine diatoms is about 16:16:1 in

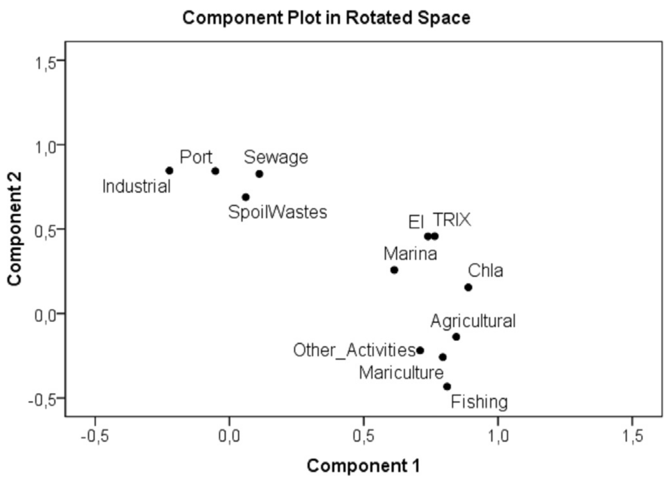

Fig. 3. Distribution of the pressures and eutrophication assessment results, within the new multidimensional space defined by the factor analysis (rotated, using the Varimax rotation method) in the euphotic zone.

a nutrient-replete ecosystem (Redfield, 1958; Xu et al., 2008). Deviation from the Redfield ratio indicates the potential for N, P, or Si limitation of phytoplankton growth. In our assessment of stoichiometric limitations, we have calculated Redfield ratios following Pavlidou et al. (2004) and Xu et al. (2008) to predict

1. N limitation occurs when DIN:P <16 and DIN:Si <1;
2. P limitation occurs when DIN:P >16 and Si:P >16;
3. Si limitation occurs when DIN:Si >1 and Si:P <16.

The data indicated significant probable P-limitation (69%), whereas N-limitation was found at the 31% of the cases and Si-limitation only at 7%. Among the study coastal areas, Saronikos, Thermaikos, and Amvrakikos gulfs indicated N-limitation.

Changes in DIN:P:Si ratios may produce "undesirable disturbances" (e.g. the potential effects of increased production and the direct and indirect changes in the balance of organisms) on the ecosystem structure and function, as well as on the ecosystem goods and services used by humans. However, such effects do not always result from nutrient enrichment and may be triggered by other causes (Ferreira et al., 2011), while there is still a lot of discussion on how and which useful nutrient ratios are assessed in eutrophication studies (Newton et al., 2003). In our case, the relatively lower DIN:P ratios were recorded in coastal areas where some of the direct and indirect effects of eutrophication have been recognized. More specifically, in Amvrakikos gulf hypoxic and anoxic conditions occurred, while in Thessaloniki bay (Thermaikos gulf) increased abundance of certain harmful dinoflagellate species was recorded. Indeed, in Thermaikos gulf, dinoflagellates were the dominant blooming species from 1996 onwards including the toxic species *Dinophysis acuminata* (Reizopoulou et al., 2004; Kouhypoxikaras and Nikolaidis, 2004; Pagou, 2005; Vakirtzi et al., 2006, 2010).

The nutrient ratio DIN:P correlated negatively and significantly (at level 0.05) with the pressure index, indicating that the increase of the anthropogenic pressures results in more N-limited coastal areas. In addition, the mean integrated DIN:P ratio calculated for the whole water column showed significant negative correlation (at the level 0.05) with mariculture, agriculture pressures (correlation coefficients: -0.391 and -0.455, respectively) and with other pressures (at level 0.01; correlation coefficient: 0.519) whereas it did not correlate with sewage and industrial discharges and port activities.

The mean integrated DIN:Si ratio correlated positively and significant (at level 0.01) with industrial pressures (correlation coefficient: $+0.636$). In addition, DIN:Si ratio correlated negatively and significant (at level 0.01) with fishing and other activities (correlation coefficients: -0.638 and -0.632, respectively), whereas, at level 0.05 DIN:Si ratio correlated negatively and significant with mariculture (correlation coefficient: -0.403). Si:P ratio correlated negatively and significant (at level 0.05) with sewage discharges. According to those correlations, heavy industrial pressure and sewage discharges would result to the increase of DIN:Si ratio and decrease of Si:P ratio, thus potentially to the shift of the ecosystem towards Si-Limitation, whereas heavy pressure from agriculture, mariculture and fishing activities would potentially lead to the shift of the ecosystem towards N-Limitation.

It seems that at the selected coastal Greek areas, deviation of Si:DIN:P ratios from the theoretical value are probably related mainly to the mariculture, agriculture, fishing, industrial and sewage discharges.

A factor analysis (FA) among the pressures, the eutrophication indices (E.I. and TRIX) and the nutrient ratios was also performed for the entire water column data treatment (Figure is not presented). The analysis resulted to three main factors or components (71% of total system variability was explained; 36% by the first factor, 23% by the second, and 12% by the third). According to this analysis, mariculture, agriculture, fishing and other activities together with the eutrophication indices acquired the highest loadings in the first component, sewage and industrial discharges together with DIN:Si ratio acquired the highest loadings in the second component, while in the third component the DIN:P and Si:P ratios acquire high loadings.

Among the N-limited coastal areas, the lowest mean integrated DIN:P and DIN:Si values (4.8 and 0.05, respectively) together with the highest Si:P (177) value were recorded in South Amvrakikos gulf, where anoxic condition occurred. Thus, this area with high eutrophication problems was characterized by excess of silicate and nitrogen depletion probably due to its consumption by phytoplankton or other biochemical processes (e.g. denitrification). The mean integrated value of phytoplankton biomass in South Amvrakikos gulf was 3.00 mg m^{-3}. On the other hand the highest DIN:P and DIN:Si and Si:P values were recorded in Patraikos gulf, an area with light anthropogenic pressure. Limnos with very minor anthropogenic impact is characterized by relatively high mean integrated DIN:P, DIN:Si and Si:P values (54, 0.28 and 176, respectively), indicating surplus of inorganic nitrogen and silicate in this phosphorus limited area. The mean integrated values of phytoplankton biomass in Limnos island and Patraikos gulf were 0.05 and 0.19 mg m^{-3}, respectively.

Skogen et al. (2004) have used DIN:P ratio as a possible proxy for eutrophication and have proposed five different classes of DIN:P. According to this classification scheme, west and south Patraikos, Limnos island, Patra, Korinthos and Elefsis bay (S1) are grouped together with very high and high mean integrated DIN:P ratio values (50–100 and >100) and low phytoplankton biomass concentration (0.18 ± 0.08 mg m^{-3}). These are areas characterized by low impact of maricultures, agriculrure and fishing. On the other hand, areas with high and heavy anthropogenic pressures, mostly mariculture and agricultural, are grouped together with low and/or very low (16–30 and <16, respectively) mean integrated DIN:P ratio values and higher concentrations of phytoplankton biomass (1.50 ± 0.99 mg m^{-3}). Unfortunately, the lack of data on composition and abundance of the phytoplankton communities do not allow us to investigate and discuss any differences on the ecosystems structure and function.

3.3. Eutrophication assessment with different methodological tools

Few methods have been developed in the EU in order to assess eutrophication and evaluate its trends. The state of the art in

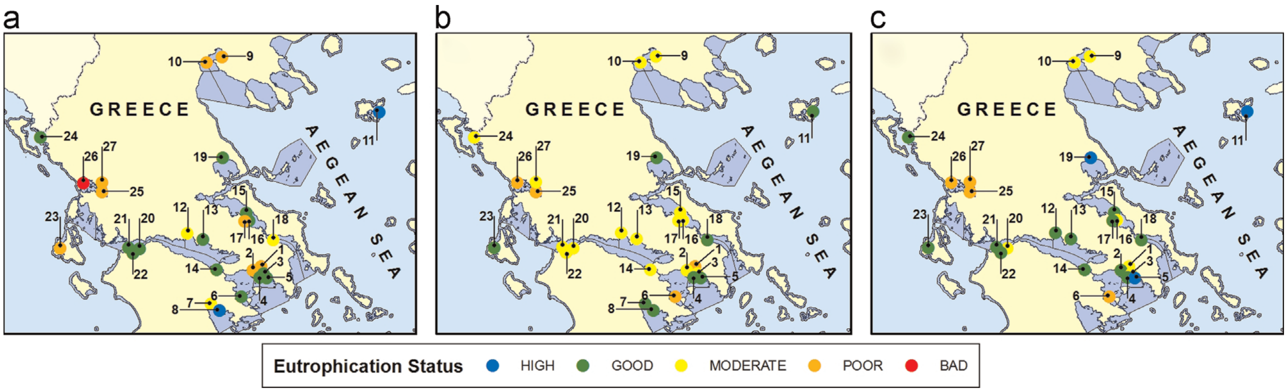

Fig. 4. Eutrophication assessment of the Greek coastal water bodies according to chlorophyll-a, TRIX, and E.I. applied in the entire water column.

Greece, regarding the eutrophication assessment of the coastal waters, is the following: A Greek eutrophication scale was developed by Ignatiades et al. (1992), Karydis (1999) and Pagou et al. (2002), and has been used extensively ever since. It involved four levels of trophic status, thus, in order to fit the five step ecological status scale of WFD, chl-a values from the Greek eutrophication scale were modified by Simboura et al. (2005). The boundaries of this scale were decided during the EU WFD 2nd intercalibration phase and published in the "Commission Decision of 20 September 2013 establishing, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, the values of the Member State monitoring system classifications as a result of the intercalibration exercise and repealing Decision 2008/915/EC.

In addition, two multiparametric methods, E.I. and TRIX, were used for the assessment of the trophic conditions of the Greek coastal waters, according to the WFD requirements. The boundaries of TRIX have been modified by Primpas et al. (2010) in order to be used for the oligotrophic waters of the Eastern Mediterranean.

Phytoplankton is usually employed as an indicator of change in nutrient loads and as a key element for assessing eutrophication in marine systems. Indeed, its assessment has been required by different legislations (Borja et al., 2012; Garmendia et al., 2013), and has also been applied in this study together with E.I. and TRIX indices. Figs. 4 and 5 present the eutrophication assessment according to the three different methodological tools applied in Greece, (a) for the whole water column of the study water bodies and (b) for the euphotic zone. Euphotic zone reaches to 1.5 m in the inner Thermaikos gulf to 87 m in Western Saronikos gulf. The estimation of the euphotic zone (Welch, 1948) is three times the Secchi disk disappearance depth. Also, Simboura et al. (2015) analyzing the variance of the Secchi disk disappearance depth in relation to ecological status over Greek coastal waters, found that the moderate to good threshold limit is located at 11 m depth. This means that on average and in relatively unpolluted waters, the euphotic zone may extend to 30–33 m depth.

The eutrophication assessment, when using data from the whole water column of the monitoring stations, showed that according to the E.I. 30% of the studied coastal stations were characterized as in good eutrophication status, 55% in moderate status, and 15% in poor status, while 0% of the stations were classified into high or bad status. On the contrary, according to the TRIX index tool, 56% of the monitoring stations were assessed into the high eutrophication status, 28% into good status, 12% into moderate status, 4% into poor status, and 0% into bad status. The eutrophication assessment according to phytoplankton biomass expressed as chl-a concentrations, showed that only 7% of the monitoring stations were classified into the high eutrophication status, 48% into good status, 11% into moderate status, 30% into poor status, and 4% into the bad eutrophication status. It is obvious that there is a certain degree of divergence between the resulting classifications from the different methodological tools used for the assessment of eutrophication in Greece. Thus, low class agreement between E.I and TRIX has been observed (Table 4).

When we used data from the euphotic zone only, the eutrophication assessment showed that according to E.I. 54% of the monitoring stations were classified into good eutrophication status, 35% into moderate status, 11% into poor status, and 0% into high and bad status. According to TRIX, the eutrophication status was rather upgraded with 29% of the stations classified into high status, 54% into good, 17% into moderate, and 0% into poor or bad status. According to chl-a criterion, the classification was more severe, with only 4% of the monitoring stations classified into high status, 50% into good status, 11.5% into moderate status, 23% into poor status, and 11.5% into bad status. In the euphotic zone E.I. and TRIX showed low agreement also (Table 4).

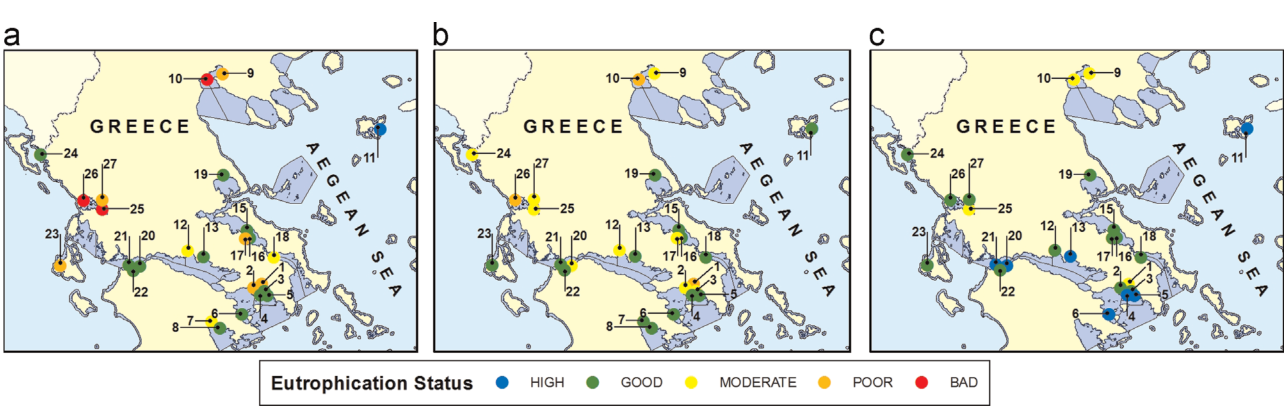

Fig. 5. Eutrophication assessment of the Greek coastal water bodies according to chlorophyll-a, TRIX, and E.I. applied in the euphotic zone.

Table 4
Kappa values and agreement, matching and mismatching percentage, Pearson correlation between the different methods (a) entire water column,(b) euphotic zone.

	Chl-a		E.I.		TRIX	
	Kappa value	Pearson	Kappa value	Pearson	Kappa value	Pearson
a) Entire water column						
BENTIX	0.43 Moderate	−0.7155**	0.44 Moderate	−0.4986**	0.31 Low	−0.4832*
EEIc	0.6 Good	−0.7189**	0.38 Low	−0.3074 0.1874	0.23 Low	−0.3367 0.1718
E.I.	0.4 Moderate	0.5528**			0.31 Low	0.8937**
TRIX	0.28 Low	0.5373**				
b) Euphotic zone						
BENTIX	0.39 Low	−0.7222**	0.47 Moderate	−0.6289**	0.23 Low	−0.6686**
EEIc	0.68 Good	−0.7993**	0.47 Moderate	−0.5452*	0.31 Low	−0.6800**
E.I.	0.62 Good	0.8693**			0.38 Low	0.7171**
TRIX	0.32 low	0.7143**				

* Significant correlation at 0.05 level.
** Significant correlation at 0.01 level.

According to MSFD, the indirect effects of eutrophication are abundance of perennial seaweeds and sea grasses impacted by decrease in water transparency and DO changes, for example, hypoxic or/and anoxic events as well as changes in macrozoobenthic species composition (Ferreira et al., 2010; Borja et al., 2013). In our cases, hypoxic or/anoxic events have been recorded in S. Amvrakikos gulf and the deep layer of the western Saronikos gulf, whereas relatively lower oxygen concentrations have also been recorded in Elefsis bay and Thessaloniki bay. In addition, decrease in water transparency has been observed in Amvrakikos gulf and Thessaloniki bay, but not in the western Saronikos gulf (Table 1), whereas in Thessaloniki bay, harmful algal blooms have been developed. According to this information, these areas reflect some of the direct and indirect effects of eutrophication in the water column. South Amvrakikos and western Saronikos gulf have been classified into poor status using both E.I. and TRIX.

Oxygen deficiency can result from the sinking and decomposition of the excess organic matter produced as a result of eutrophication, but it can also be the result of other causes, including decreases in the ventilation of deep water caused, for example, by climate change. This is the case of the western Saronikos gulf with high residence time of the deep water layer. On the other hand, in Amvrakikos gulf nutrients are introduced into the surface layer of the semi-enclosed water body of Amvrakikos gulf from riverine discharges resulting to water column stratification and oxygen deficiency in the near bottom water layer. These examples indicate the significant role of hydrodynamics, bathymetry, sedimentary processes, and so on in the eutrophication assessment (Ferreira et al., 2010; Newton et al., 2014). Amvrakikos and western Saronikos gulfs, which have been classified into poor status with both E.I. and TRIX in the whole water column, reflect some of the direct and indirect effects of eutrophication in the water column, which affect also the perennial seaweeds and sea grasses (Ferreira et al., 2010; Newton et al., 2014). However, according to Garmendia et al. (2012), the inclusion of secondary indicators (e.g. lower DO levels, appearance of HABs, or changes on the benthic community) in assessment methods is very important and provides a more robust picture with a better perspective of the scale of the eutrophication problem than methods that consider only water-column chemistry. Thus, the assessment methods that consider only primary indicators of the water column may downgrade the eutrophication status of an area, as they do not include secondary indicators. Including additional parameters for the eutrophication assessment (e.g. D% O_2 in TRIX) is in general useful, but it can also bias the eutrophication status because it can be related to other pressures or factors (such as climate change) different from nutrients pressure (Garmendia et al., 2012).

According to Devlin et al. (2011), TRIX method, which uses a combination of N, P, phytoplankton, chl-a, and DO saturation may produce biased results because it assumes that eutrophication processes are mainly reflected as changes in phytoplankton biomass, but this does not hold true for some ecosystems, for example, shallow systems where other primary producers (e.g. macroalgae, seagrasses, etc.) may contribute a significant amount to total primary production. The E.I. method uses the same assumption as the TRIX method as using a combination of N, P, and chl-a without though including oxygen saturation. Thus, it may also introduce a bias in the results because oxygen and probably other primary and/or secondary parameters are not included (Garmendia et al., 2012). The juxtaposition and comparison with other biological indicators (e.g. macroalgal abundance and benthic macroinvertebrate communities) may provide a more complete overview within the context of WFD. For this reason, we have examined the class agreement relations among eutrophication and benthic indices presented below.

3.4. Class agreement between indices

The E.I. and chl-a parameter are in good agreement in the euphotic zone and in moderate agreement in the entire water column data treatment, whereas the agreement between TRIX and chl-a is low in both cases. In addition, the class agreement of E.I. to the macroalgae index EEIc is low in the euphotic zone and moderate in the entire water column, whereas the class agreement of TRIX with EEIc is low at both cases of data treatment. According to this, we could assume that the E.I. probably reflects in a better way than TRIX the "direct effect" of nutrient enrichment, which is the increased primary production indicated as increased chl-a and/or macroalgal abundance. Indeed, the increase of primary production has been recognized as the biological response of nutrient enrichment in the water column (Ferreira et al., 2007; Borja et al., 2008; Ferreira et al., 2011). In that way, among the two multiparametric indexes (E.I. and TRIX), E.I. seems to have a better performance than TRIX and reflects in a more efficient way the first stages of eutrophication. However, this must be verified with other biological indices according to the class agreement analysis presented below. Different quality elements address different pressures, but WFD requires the integrated impact from all pressures (Caroni et al., 2013). The comparison among the eutrophication indices and the benthic indices aims to demonstrate the differential sensitivity of the various indices to pressures with the overall goal to indicate the most responsive tool to the sum of pressures.

Class agreement of eutrophication indices with EcoQ benthic indices was checked with data from the whole water column and with data from the euphotic zone (considered as three times the Secchi Disk depth; Welch, 1948), in order to examine the levels of linkage or relevance of the eutrophication assessment tools with the EcoQ (Table 4) following, in both cases, the weighted Kappa analysis. The Pearson atation coefficient and p values among the eutrophication indices (chl-a, E.I., and TRIX) and the benthic EcoQ indices (BENTIX and EEIc) are also presented in Table 4. According to the results for the whole water column, from the surface layer to the near-bottom layer, the eutrophication indices E.I. and TRIX show a better correlation with the BENTIX than with EEIc (Table 4). More specifically, chl-a parameter correlated positively and significantly with all eutrophication indices and significantly negatively with the EcoQ indices. The E.I. correlated positively with chl-a parameter and TRIX and negatively with the BENTIX, but it did not correlate with EEIc. The TRIX showed a significant and negative correlation with the BENTIX and a positive one with chl-a and E.I. The BENTIX and the EEIc indices correlated with each other significantly. According to the above, among the eutrophication indices and criteria, chl-a showed linear correlation with both EcoQand in good agreement with EEIc. Moreover, E.I. and TRIX eutrophication indices correlated significantly only with the BENTIX, noting that E.I. had stronger correlation and better agreement with BENTIX than TRIX had.

However, for the euphotic zone chl-a showed good agreement with EEIc and low with BENTIX. Regarding E.I., the class agreement was the same (moderate) both with BENTIX and EEIc. Pearson correlations in the euphotic zone treatment of data are all significant (Table 4).

In general, for both cases of data treatment, we observed that class agreements and correlations between TRIX and benthic indices were at all cases low. The class agreement between chl-a and TRIX was always also low (Table 4). EEIc linked better with chl-a in both cases of treatment (good agreement) because macroalgae and chl-a are both primary producers representing the same level of the trophic web. Macroalgal growth and chl-a increase are “direct” effects or “primary symptoms” indicating the first stages of eutrophication. It is noteworthy that among the eutrophication indices, E.I. showed overall better (moderate) agreement with BENTIX. Macroinvertebrates are secondary producers and the “indirect” effects of eutrophication. This means that we expect no direct linkage between water eutrophication (nutrients) and benthic macroinvertebrates because it flows via plant growth and organic compounds. However, there is an indirect relation of macroinvertebrates with water eutrophication, which is better reflected to E.I. (moderate agreement).

The coastal macroalgae through the index EEIc are more sensitive in capturing the effect of the surface or euphotic zone eutrophication, expressed through chlorophyll biomass levels.

From the above, it seems that among BENTIX and EEIc there is a divergence concerning the relationships with eutrophication indices. Indeed, at many cases of coastal and transitional water bodies, macroalgae and chl-a biomass have produced a high disagreement with the macroinvertebrates assessment, and consequently, with the integrative assessment that assigns a special weightage to benthic communities as being good indicators of environmental quality (Borja and Rodríguez, 2010). The increased reliability of a given method was based on the assumption that the method or index is used broadly by authors other than the proposers of the method, was tested for several different human pressures, and/or intercalibrated with other methods. Indeed, the intercalibrated BENTIX was designed for the Mediterranean benthic ecosystem and has been tested using various anthropogenic pressures, such as eutrophication and organic pollution, mining residues, and aquaculture in Greece, Cyprus, and the Western Mediterranean through the intercalibration exercise (GIG, 2013; Simboura et al., 2012 and references within).

The observed mismatching has been attributed to the high spatial and temporal variability of some of the biological elements, such as the macroalgae and phytoplankton (Borja et al., 2004). A similar analysis of WFD data originating from the implementation of WFD in Greece also verifies this divergence between macroalgae and the benthic element, and the general EcoQ and attributes this to the specific sensitivity of macroalgae to the point source pressures exerted on the coasts of a water body (Simboura et al., 2015). The later effect of the localized direct effect of eutrophication on the coast is getting more evident in the present work.

Caroni et al. (2013) showed that the sensitivity of the different biological quality elements (BQEs) to various pressures influences the confidence level and comparability of the various methods for combining the assessment results. The BQEs used for the WFD assessment may be sensitive to the same pressure, be complementary in displaying the effects of different pressures, on different spatial and or temporal scales, on different aspects of the ecosystem functioning, or be responsive to multiple pressures.

Integrating both aspects of eutrophication including primary and secondary effects such as macroalgae, benthic macroinvertebrate communities, and water eutrophication indices through the application of an integrative method such as decision tree (Simboura et al., 2015) will ensure the capturing of all eutrophication effects on ecological and environmental status assessments.

4. Conclusions

In this work, the eutrophication status of selected Greek coastal areas was studied under various pressures using different indicators and different methodological approaches. The methods applied for Greek coastal areas (TRIX, E.I., and chl-a biomass criterion) did not give absolutely matching results of eutrophication assessment for all the studied water bodies.

Agricultural activities, mariculture, fishing, and other activities (e.g. riverine discharges) are the pressures that mostly related to the eutrophication status assessment methodologies used for the selected Greek coastal water bodies.

Chl-a linked better with EEIc in both cases of treatment (good agreement). It is noteworthy that among the eutrophication indices, E.I. showed overall better (moderate) agreement with BENTIX.

Chl-a is the most relevant indicator to reflect eutrophication impact on macroalgae. Moreover, E.I reflects better the indirect relation of eutrophication.

The evaluation of the eutrophication status applied in dynamic coastal areas depends on the selected indicator as each one focuses on different aspects of the ecosystem (concentration of nutrients, DO, chl-a, benthic, and planktonic organisms). In Mediterranean, and especially in the oligotrophic Eastern Mediterranean, it is still not well established which physicochemical and/or biological indices should be used. Among the methods used in this study, chl-a reflected more efficiently the first stage of eutrophication (macroalgae), while E.I. reflected better the integral eutrophication status of a water body as a whole. In the integrative status implementation of Greek coastal waters, E.I. has been used as a surrogate for the general physicochemical status used together with the biological elements following the decision tree of Borja et al. (2009) that gives a special weightage to the macroinvertebrates (Simboura et al. 2015). Therefore, it could be considered as a reliable tool with regard to the assessment of eutrophication status, as well as, to the implementation of nutrient management strategies under the EU WFD and the EU MSFD. Nevertheless, it should be stressed that the station in Limnos Island (very minor pressures) has been classified into high status according to the TRIX, chl-a parameter and benthic indices, but into good status according to E.I. According to this, we could preliminarily assume that probably the E.I. is too sensitive or stringent, resulting in the downgrade of the quality status of Greek sites corresponding to minor pressure index values and a future modification of the high to good boundary of E.I. may be needed in order to demonstrate the high status of some relatively undisturbed Greek coastal sites.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.csr.2015.05.013.

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