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Assessment of trophic status of the northeastern Mediterranean coastal waters: eutrophication classification tools revisited

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Abstract

The Eastern Mediterranean and its Cilician Basin offshore waters have oligotrophic features with low nutrient concentrations, low primary production, and high water transparency. However, the wide shelf area of the Cilician Basin is subject to contaminated river inflows with enhanced nutrient loads and direct discharges of urban wastewaters of southern Turkey, leading to develop local eutrophic/mesotrophic conditions in the inner sites of Mersin and Iskenderun Bays on the Cilician Basin. For the assessment of changing trophic status of the coastal and the bay water bodies under anthropogenic pressures since the 1980s, five extensive field studies were performed in summer and winter periods of 2014, 2015, and 2016. Physical and eutrophication-related biochemical parameters (salinity, temperature, Secchi Disk Depth, nutrients, dissolved oxygen, chlorophyll-*a*) were measured at 65 stations in different water bodies occupying the Northeastern (NE) Mediterranean coastal, offshore areas and bays. The collected data sets were used in scaling the trophic status of the visited water bodies of NE Mediterranean coastal, offshore areas and semi-enclosed bays, using novel classification tools of Trophic Index (TRIX), Eutrophication Index (E.I.), chl-*a*, and HELCOM Eutrophication Assessment Tool (HEAT), developed by different experts for highly productive seas. These tools, which can successfully classify highly productive coastal water masses under human pressures, and their sensitivities have been tested for scaling of the current trophic status of the NE Mediterranean coastal water bodies being subject to human pressures. The scaling results of classical TRIX, E.I., and chl-*a* indices in the NE Mediterranean water masses are not sensitive enough to differentiate mesotrophic and eutrophic water bodies because these indices principally assume to have higher concentrations of eutrophication-related parameters in the least effected (reference) water bodies. The HEAT tool, which uses a site-specific "reference value" for each eutrophication-indicator, has allowed us to produce more reliable and sensitive scaling of the current trophic status of the NE Mediterranean shelf areas, even though we used only the "reference values" derived from the composite data sets. The results of the indices were compared with the HEAT tool and the actual status was assessed from observations, indicating revision requirements of the multi-metric classification tools. For this goal, scales of natural (oligotrophic) and anthropogenic (eutrophic) levels of eutrophication indicators should be determined at a sub-basin scale using long-term site-specific observations in the NE Mediterranean. The revised scale ranges of TRIX for oligotrophic, mesotrophic, and eutrophic water bodies of Mersin Bay are in line with ranges of TRIX classification tool proposed for Aegean Sea waters, which can be used to assess trophic status of the entire Eastern Mediterranean and Aegean coastal seas (surface salinity > 37.5) having oligotrophic properties in the offshore waters.

Keywords Eutrophication · Northeastern Mediterranean · Nutrients · Trophic status assessment

Introduction

The Eastern Mediterranean Sea (EM) with high saline waters on the surface layer (Fig. 1) is one of the world's oligotrophic seas due to the highly unusual anti-estuarine circulation with a residence time of surface and intermediate water of only 7–8 years, limiting nutrient inputs from internal sources as well as removing a significant fraction of nutrients supplies from terrestrial and atmospheric sources to intermediate depths

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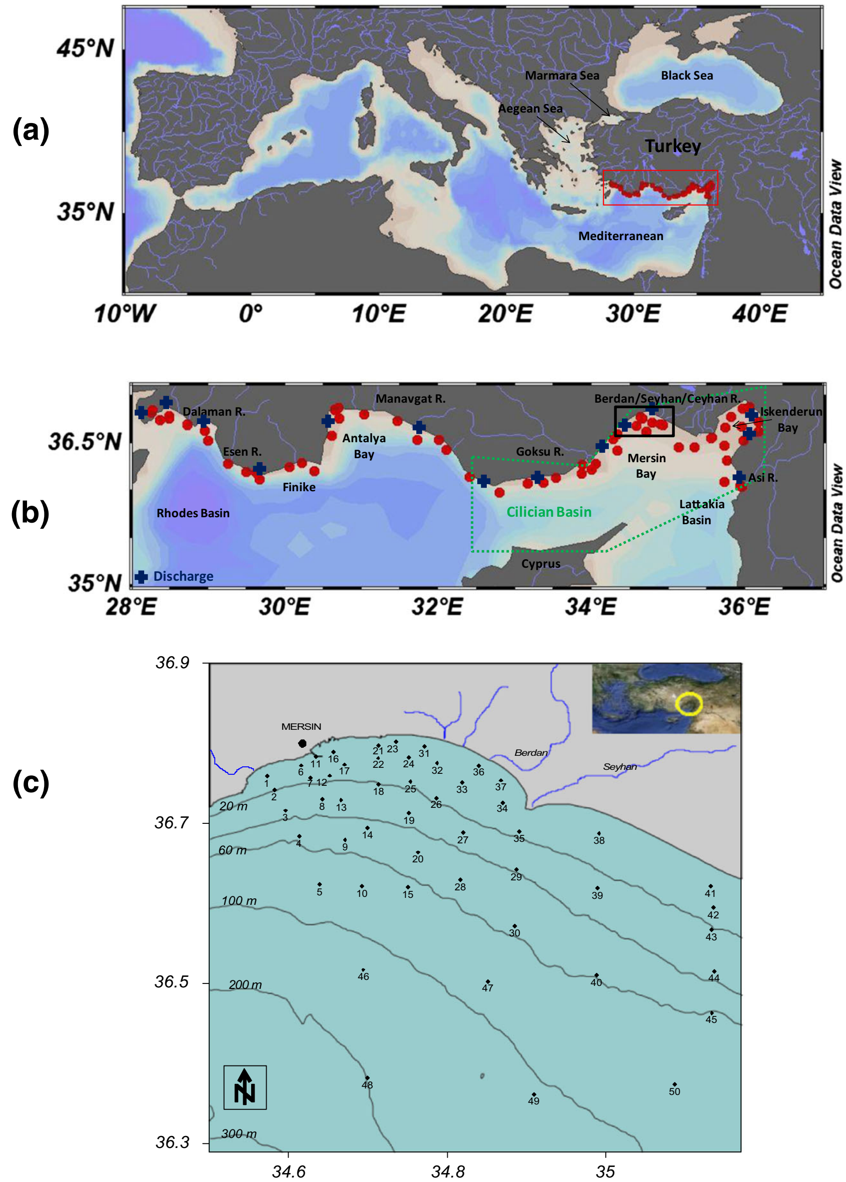

Fig. 1 **a** Geography of the Mediterranean. **b** Sampling stations visited along the NE Mediterranean in 2014–2016 period. **c** Mersin Bay Monitoring stations between 2008 and 2013

(Yılmaz and Tugrul 1998; Kress and Herut 2001; Krom et al. 2004; Kocak et al. 2010; Tanhua et al. 2013; Powley et al. 2017 and references therein). In the NE Mediterranean offshore waters, surface nutrient concentrations of PO_4 are measured at 0.02–0.03 μM and NO_3 at 0.1–0.3 μM in the period of spring-autumn including summer, increasing slightly in winter by inputs from lower layer waters via vertical mixing (Yılmaz and Tugrul 1998; Dogan-Saglamtimur and Tugrul 2004). The continental shelf waters of the Cilician Basin, Antalya Bay, and Finike-Marmaris coastal seas and bays on the NE Mediterranean in Turkey are fed by nutrient and organic matter inputs from the major rivers (Asi, Ceyhan, Seyhan, Goksu) and medium scale rivers (e.g., Berdan, Lamas, Manavgat, Aksu, Esen, Dalaman) (Fig. 1). Increased nutrient inputs have led to deterioration of water quality and development of eutrophication in the Mersin and Iskenderun Bay coastal waters having limited ventilation by open sea during late spring-autumn periods. Enhanced organic matter production in the surface waters has altered optical and biochemical properties of the inner bay waters (Tugrul et al. 2016); surface chl-*a* values have increased by more than tenfold (1.0–3.5 μg/L) compared to the offshore values of 0.05–0.1 μg/L and Secchi Disk Depth has declined to 2–5 m levels on the hot spots (Tugrul et al. 2011).

In order to evaluate the trophic status of human-impacted enclosed seas, coastal water, and bay waters, eutrophication classification tools have been developed, using the direct and indirect indicators of eutrophication in the marine environments (Vollenweider et al. 1998; HELCOM 2009; Primpas et al. 2010; Andersen et al. 2011; Ferreira et al. 2011). The major indicators of eutrophication, namely nutrients, chl-*a*, dissolved oxygen saturation level in deep water, Secchi Disk Depth, phytoplankton composition, and macro-benthic diversity changes, have been used to develop multi-metric or univariate classification scale for eutrophication assessment tools. The tools used in the Mediterranean region are Trophic Index (TRIX) developed by Vollenweider et al. (1998), Eutrophication Index (E.I.) by Primpas et al. (2010), and HELCOM Eutrophication Assessment Tool (HEAT) used by HELCOM (2009) and Andersen et al. (2011). To scale oligotrophic, mesotrophic, and eutrophic water qualities in the Mediterranean Coastal Water Bodies (CWBs), univariate scales based on chl-*a* and inorganic nutrient concentrations have been proposed by Simboura et al. (2005) and Ignatiades et al. (1992), respectively. Inorganic nutrients supplied to the surface waters are consumed in photosynthesis; therefore, surface nutrient concentrations in the marine environments increase in winter-spring period when the input rates exceed the uptake and then decrease to the background levels in the slightly disturbed coastal water bodies and undisturbed open sea during the dry and stratified periods. Since the EM surface waters have low concentrations of nutrients and biomass, the reliable and sensitive nutrient data are needed for reliable classification of the trophic status of the NE Mediterranean coastal and offshore waters under anthropogenic pressures. However, all these classification tools have some of flaws/challenges in the oligotrophic NE Mediterranean waters having P-limited or N and P-co-limited ecological properties in the coastal and open seas (Thingstad et al. 2005; Powley et al. 2017). The TRIX Index is a multi-metric method developed for the classification of highly productive coastal seas and has insufficient sensitivity for the assessment of developing eutrophication conditions in the oligotrophic NE Mediterranean, indicating the necessity of new approaches for less productive water bodies under the pressures of human-induced nutrient inputs. This study aims (1) to assess the current trophic status of NE Mediterranean shelf waters using the recently developed classification tools mentioned above using our data sets and (2) to evaluate these classification tools' sensitivities in the coastal and bay waters of NE Mediterranean. We finally suggest possible revisions in use of TRIX index in the NE Mediterranean coastal water bodies and shallow bay waters to differentiate mesotrophic and eutrophic water bodies influenced by human-induced nutrient inputs.

Materials and methods

The sampling stations selected along the Turkish continental shelf (Fig. 1) were visited in September 2014, February 2015, August 2015, February 2016, and August 2016. Principal physical and biochemical parameters were measured at about 65 stations (Fig. 1b), using R/V BILIM-2 of METU-IMS. Furthermore, current and previous data sets obtained by the METU-IMS (Table 1) from these stations were also pooled in the present measurements to increase data set of eutrophication-related parameters in coastal and offshore waters of the NE Mediterranean.

Physical parameters (temperature, salinity, density) were measured in situ by a SEABIRD model CTD probe which is coupled to a 12-Niskin bottle Rosette System. Seawater samples were collected at pre-selected depths by remote control. The Secchi Disk Depth (SDD), a rough estimation of water transparency, was measured at all the stations during the day time. Dissolved oxygen measurements were carried out by the automated Winkler titration method (Grasshoff et al. 1983; UNEP/MAP 2005). Dissolved inorganic nutrients (nitrate+nitrite, ammonium, phosphate, and reactive silicate) were determined using a Bran+Luebbe Model four-channel Autoanalyzer by standardized methods (Grasshoff et al. 1983). All the unfiltered nutrient samples kept cool in high-density Polyethylene (HDPE) bottles (not frozen) were analyzed on board during the period of 2008–2015. Then, the samples collected in 2016 were frozen without filtering and then analyzed at the METU-IMS laboratory within 2 weeks. The detection limits of nitrate+nitrite 0.04 μM, ammonium 0.04 μM, phosphate 0.01 μM, and silicate 0.04 μM. Unfiltered samples for total phosphorus (TP) were kept frozen until analysis (3–4 weeks). After thawing, the subsamples (40 mL) were digested by perfsulfate oxidation method (Menzel and Corwin 1965). After pH adjustment and reagent addition, the absorbance of each sample was measured manually by the conventional colorimetric method at 880 nm wavelength (detection limit 0.03 μM) (Strickland and Parsons 1972; Grasshoff et al. 1983). Inorganic nutrient and TP standards were prepared in the nutrient-low offshore Mediterranean surface seawater for quantification of the sample absorbance after baseline/blank corrections. Chlorophyll-*a* (chl-*a*) samples were filtered (GF/F type filters) on board, stored in the foil, and frozen till analysis (within 3–4 weeks). Chl-*a* measurements were carried out by the conventional spectrofluorometric method after digestion of filtered samples by 90% acetone solution, using a HITACHI model F-2500 Fluorescence Spectrophotometer (Strickland and Parsons 1972; UNEP/MAP 2005).

Results and discussion

Spatial distributions of physical and eutrophication-indicator biochemical parameters

For the assessment of current eutrophication status of the NE Mediterranean shelf and bay waters, surface water distributions (0–10 m averages) of eutrophication-related parameters were determined at about 65 stations (Fig. 1b) in the winter and summer months of 2014–2016 period (see Table 1). Surface distributions of physical and biochemical parameters

Table 1 Study areas and periods (season/month) of data sets collected in the NE Mediterranean

Study area	Study period	Season
Mersin Bay	2008–2013	Autumn (October, November)
		Winter (January, February, March)
		Spring (April, May)
		Summer (July, August, September)
NE Mediterranean shelf and bays	2014–2016	Summer (August–September)
		Winter (February)

are depicted in Fig. 2. Surface salinity, an indicator of freshwater inflow, varied from 37.1 in the river-fed coastal zone to 39.6 in the offshore waters in summer. The lower salinity values, ranging between 37.1 and 38.0, were determined in the Mersin and Iskenderun Bay shelf waters fed by Ceyhan and Seyhan River inflows. The maximum salinity values were consistently recorded in the offshore waters of Cilician Basin shelf in August–September (Fig. 2). Secchi Disk Depth (SDD) values, an indicator of water transparency, were measured to range from 1.0–3.0 m in the less saline shallow zone of Mersin Bay to 30–36 m levels in the high saline nutrient depleted open sea of the NE Mediterranean in August–September (Fig. 2); these findings are highly consistent with previous results from the Cilician Basin shelf waters including Mersin Bay (Dogan-Saglamtimur and Tugrul 2004; Tugrul et al. 2011).

Surface water concentrations of dissolved inorganic nutrients also displayed quite high regional variations in the NE Mediterranean shelf and bay waters (Table 2); the peak values were recorded in the river-fed coastal surface waters and inner bay waters receiving domestic wastewater discharges (Fig. 2). As shown in Table 3, the major rivers (Seyhan, Ceyhan, Goksu) in the Cilician Basin introduce large amounts of NO_x (nitrate+nitrite) and reactive Si inputs annually; their nutrient loads much exceed the inputs by wastewater discharges and influence nutrient cycling in the inner bay and shelf waters (Kocak et al. 2010). However, the phosphorus loads of the rivers are markedly low, leading to P-limited primary production in the river-fed shelf waters (Tufekci et al. 2013). The surface NO_x concentrations were as low as 0.04–0.1 μM in more saline offshore waters during the late summer months; higher values were determined in the less saline coastal zone, ranging between 2 and 4 μM (Fig. 2). The extremely high values (> 20 μM) of NO_x were obtained in the estuarine waters (depth < 10 m and S < 37.5) of Ceyhan and Goksu deltas in the wet winter month (February). Surface layer PO_4 concentrations expectedly displayed similar but less pronounced spatial variations, ranging from 0.02 μM in the oligotrophic offshore waters to 0.3 μM in the nearshore zone receiving urban wastewater discharges (Fig. 1b marked by +). Total phosphorus (organic+inorganic-P) concentrations ranged from 0.06–0.1 μM in the offshore to 0.2–0.85 μM in the river-fed coastal waters (S < 38.5) and shallow inner bay waters polluted by domestic wastewater discharges. Spatio-temporal distributions of the surface NH_4, ranging between 0.05 and 4.7 μM, were similar to the phosphorus pattern, elevated concentrations (> 10 μM) at the hot points within the Iskenderun bay receiving anthropogenic inputs and surface runoff in winter (Fig. 2). Surface layer averages of reactive Silicate (Si) concentrations were as low as 0.50 μM in the offshore in summer and increased over 20 μM in the estuarine waters fed by Ceyhan and Goksu rivers in the wet winter period (Table 2). Nutrient data obtained in the regional rivers flowing to Cilician Basin wide shelf (Table 3) indicate that the Si and NO_x concentrations in the major rivers ranged between 80 and 150 μM as previously reported elsewhere (Dogan-Saglamtimur and Tugrul 2004; Kocak et al. 2010; Tugrul et al. 2011, 2016). However, enhanced DIN inputs to the dammed river inflows of Cilician Basin has altered Si/NO_x ratio (< 1.0) in freshwaters and thus coastal waters fed by these rivers.

The highest concentrations of chl-*a* and nutrients were observed in the coastal waters fed by river inflows and the coastal zone under human pressures (Fig. 2, Table 2). Markedly high chl-*a* values, reaching maximum values at 3–3.5 μg/L, were recorded in the previous studies in the Mersin inner bay productive waters (Tugrul et al. 2011). More saline coastal water bodies have markedly low chl-*a* values, very similar to the oligotrophic offshore properties (Yılmaz and Tugrul 1998; Kress and Herut 2001; Krom et al. 2004).

Surface waters have saturated levels of dissolved oxygen (DO); the saturation level was slightly higher (102–104%) in the more productive coastal/bay waters fed by nutrient-laden river inflows (Fig. 2). The summer DO concentrations (5.8–7.1 mg/L) are always less than the winter values (7.4–8.2 mg/L) due to cooling of surface waters in winter. No oxygen deficiency was recorded in the bottom waters in the NE Mediterranean shelf. The oxygen saturation (%) barely decreased seasonally to 75–80% levels in the Iskenderun inner bay bottom waters having limited oxygen inputs by mixing from surface layer and exchange rates with open sea during the summer-early autumn period. In winter, however, the seasonal thermal stratification disappears and the entire water column down to 150–200 m in the shelf gets saturated in oxygen by intensive vertical mixing processes. In the deep basin (depth > 200 m), however, DO content of the

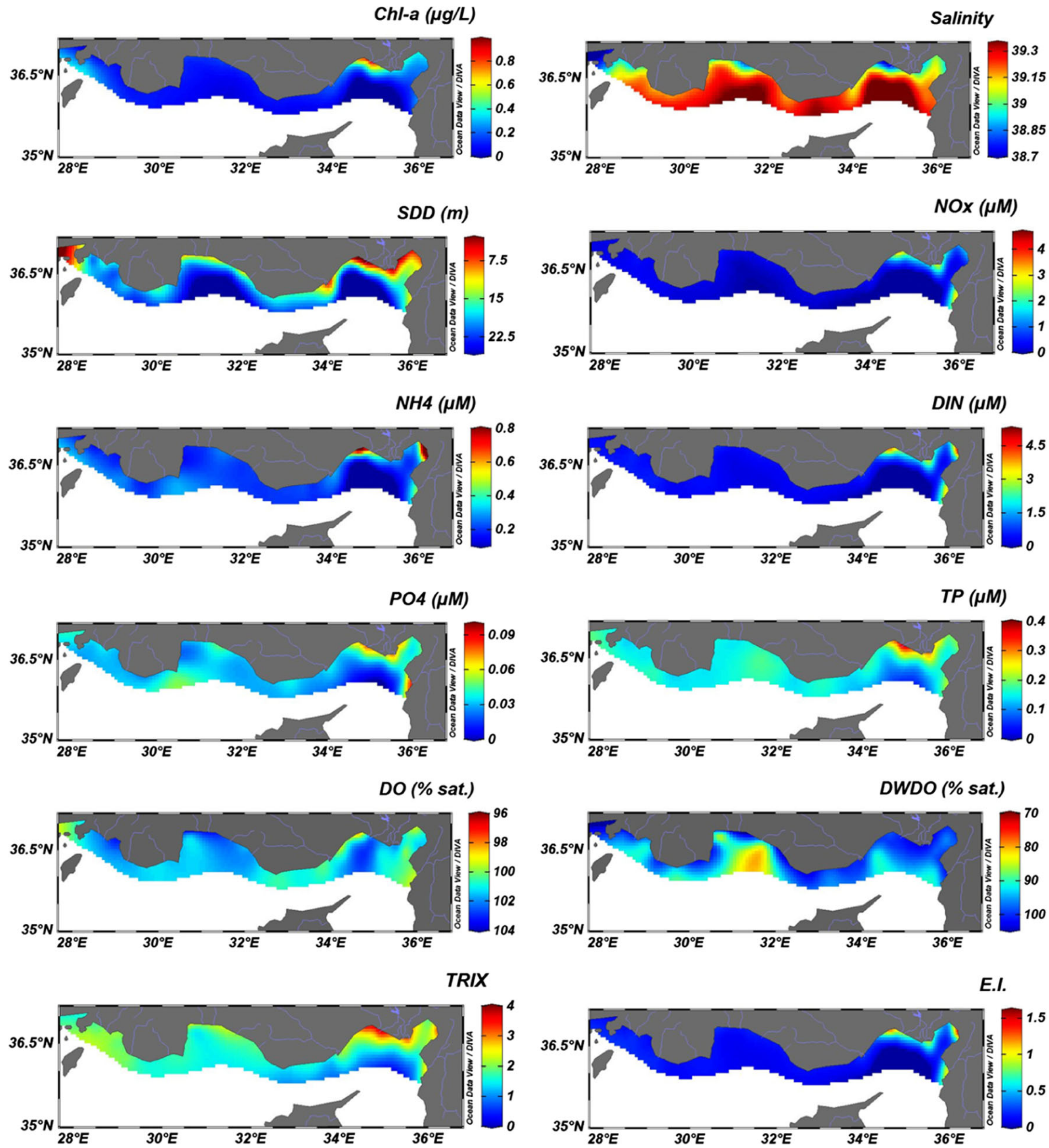

Fig. 2 Regional variations of 3-year surface layer averages (0–10 m data sets of the 2014–2016 period) of chlorophyll-*a* (chl-*a*), salinity, Secchi Disk Depth (SDD), nitrate+nitrite (NO_x), ammonium (NH_4), dissolved inorganic nitrogen (DIN: $NH_4 + NO_x$), phosphate (PO_4), total phosphorus (TP), surface and deep water oxygen saturation (%) levels, and TRIX and E.I. indices in the NE Mediterranean shelf areas and bays

Mediterranean deep layer waters remains at saturation levels of 70–75% due to the limited ventilation of deep basin by convective mixing (Yılmaz and Tugrul 1998).

Assessment of trophic status of the northeastern Mediterranean coastal waters using different eutrophication classification tools (TRIX, E.I., chl-*a*, HEAT)

For the assessment of trophic status of the NE Mediterranean shelf waters and semi-enclosed bays (Iskenderun, Mersin, Fethiye, Marmaris*)*, the sensitivity and applicability of the four different classification tools (TRIX, E.I., chl-*a*, HEAT) were examined. For this purpose, TRIX and E.I index values were calculated for the present data sets obtained in summer (August–September) and winter (February) periods using the index equations given as footnote in Table 2; the ranges of TRIX and E.I. indices determined by different experts for the NE Mediterranean coastal seas and bays are compiled respectively in Table 4. The classical TRIX has been widely used in the highly productive seas in the last two decades, but adopting modified scales in the less productive enclosed seas

Table 2 The surface layer (0–10 m) average values of eutrophication-related parameters measured in the coastal and offshore NE Mediterranean for the summer and winter periods of 2014–2016

Summer	Salinity	TP (μM)	PO_4 (μM)	NO_x (μM)	DIN (μM)	Si (μM)	DO (% sat.)	DWDO (% sat.)	Chl-*a* (μg/L)	SDD (m)	TRIX*	E.I.**
Mean	39.24	0.18	0.04	0.30	0.83	1.59	102.0	98.5	0.19	15.6	1.95	0.27
Std. dev.	0.24	0.08	0.02	0.46	3.39	1.24	3.2	8.5	0.25	8.1	0.78	0.94
Min.	38.31	0.06	0.02	0.04	0.13	0.50	86.1	68.5	0.02	1.0	0.38	0.06
Max.	39.61	0.65	0.21	4.35	46.72	7.98	109.6	111.9	1.75	36.0	4.80	12.92
N	196	194	196	196	196	196	196	196	195	191	194	196
Winter	Salinity	TP (μM)	PO_4 (μM)	NO_x (μM)	DIN (μM)	Si (μM)	DO (% sat.)	DWDO (% sat.)	Chl-*a* (μg/L)	SDD (m)	TRIX*	E.I.**
Mean	38.85	0.16	0.04	1.22	1.51	2.52	100.3	98.1	0.26	11.88	1.98	0.47
Std. dev.	0.39	0.09	0.03	3.01	3.13	3.12	1.6	5.1	0.22	6.42	0.81	0.84
Min.	37.09	0.08	0.02	0.06	0.21	0.62	97.6	70.6	0.08	0.10	0.41	0.09
Max.	39.18	0.85	0.29	24.50	25.24	24.07	106.6	106.2	1.53	28.00	4.75	6.73
N	128	128	128	128	128	128	128	128	128	125	128	128

*TRIX = [$\log_{10}$ ([TP] × [DIN] × [chl-*a*] × A%DO) + 1.5]/1.2 (Vollenweider et al. 1998)

[TP] total phosphorus (TP), *[DIN]* dissolved inorganic nitrogen (DIN) (μg/L), *[chl-a]* chlorophyll-*a* (chl-*a*; μg/L), *A%DO* absolute deviation of DO measured from the saturation condition in %

**E.I. = 0.279 × [PO_4] + 0.261 × [NO_3] + 0.296 × [NO_2] + 0.275 × [NH_3] + 0.214 × [chl-*a*] (Primpas et al. 2010)

[PO_4] phosphate, *[NO_3]* nitrate, *[NO_2]* nitrite, *[NH_3]* ammonia (μM), *[chl-a]* chlorophyll-*a* (μg/L)

of the NE Mediterranean (Primpas and Karydis 2011; Kaptan 2013). The multi-metric E.I. index in Table 2 has been proposed by researchers Primpas et al. (2010) as an alternative tool for western and central Mediterranean coastal areas having less productive water masses in the offshore reference areas.

Trophic Index

Ranges and averages of TRIX values calculated for summer/winter periods of 2014–2016, using the conventional TRIX equation (Vollenweider et al. 1998) and surface nutrient and chl-*a* concentrations and surface oxygen saturations (%) obtained at 65 stations of 22 water bodies determined in the NE Mediterranean between 2014 and 2016, are depicted in Table 2. The TRIX values varied regionally and seasonally from 0.38–3.0 in the offshore waters and less contaminated coastal waters (summer mean salinity: 39.24 and winter mean salinity: 38.85) to 3.0–4.8 in the less saline eutrophic waters fed by terrestrial inputs. The presence of close correlations between TRIX values and all the eutrophication indicators (nutrients, TP, chl-*a*, SDD) indicates deterioration of water quality and development of eutrophication (enhanced organic matter production and less water clarity by anthropogenic inputs) at the hot spots in the Mersin and Iskenderun inner bay. Based on the classical TRIX scale, the water bodies in NE Mediterranean shelf and coastal waters exhibit "Good/Oligotrophic" water quality. However, the systematic observation results of eutrophication-related parameters indicate the development of mesotrophic/eutrophic conditions in the inner zones of Mersin and Iskenderun bays as experienced in the enclosed bays of Northern Aegean Sea (Primpas and Karydis 2011). The TRIX scale revised for less productive Aegean Sea (Table 4) and Mersin Bay water located in NE Mediterranean were also used to calculate the current trophic status of the visited coastal and offshore areas (Fig. 1). The revised TRIX index values of the coastal water bodies under human pressures are classified to have "Moderate or Mesotrophic" properties. However, water quality at limited number of polluted points in the inner part of the Mersin and Iskenderun bays displayed tendency to shift to eutrophic conditions especially in summer-early autumn period when the surface layer is thermally stratified. Renewal of the coastal waters by open sea via regional and main along shore currents is much faster than the inner bay water bodies. Therefore, the high saline coastal waters consistently have lower TRIX values (Table 2), indicating "Good or oligotrophic" status based on the revised TRIX scales in Table 4. In this study, the TRIX index values exceeded 3.0 at only 36 observations out of 322 observations (9 locations out of 65 stations) suggesting development of "Moderate" to "Poor" eutrophication

Table 3 Mean nutrient concentrations and volume fluxes of the regional rivers in the Cilician Basin (Kocak et al. 2010)

River	Si (μM)	PO_4 (μM)	NO_3 (μM)	NH_4 (μM)	Q (m^3/s)
Seyhan	117	5.6	83	16	168
Ceyhan	161	1.9	105	19	144
Goksu	112	3.3	58	7	45
Berdan	91	4.8	85	34	6
Lamas	113	0.4	101	1	3

Table 4 Ranges of the TRIX, E.I., and Chl-*a* indices proposed for the Mediterranean

Region	Adriatic Sea	Ionian Sea and Aegean Sea	Mersin Bay (NE Mediterranean)	Aegean Sea	Saronikos Gulf
Eutrophication status	Eutrophication range for TRIX[a]	Eutrophication range for TRIX[b]	Eutrophication range for TRIX[c]	Eutrophication range for E.I[d]	Eutrophication range for Chl-*a*[e]
Ultra Oligotr./high	2–4	< 1.6	< 2	< 0.04	< 0.1
Oligotrophic/good	4–5	1.6–2.8	2–3	0.04–0.38	0.1–0.4
Mesotrophic/moderate	5–6	2.8–4.0	3–4	0.38–0.85	0.4–0.6
Eutrophic/poor	6–8	4.0–5.3	4–5	0.85–1.51	0.6–2.21
Dystrophic/bad		> 5.3	5–6	> 1.51	> 2.21

[a] Pettine et al. 2007

[b] Primpas and Karydis 2011

[c] Kaptan 2013

[d] Primpas et al. 2010

[e] Simboura et al. 2005

status at limited number of hot points visited in the NE Mediterranean, using the modified TRIX scale proposed by Primpas and Karydis (2011) and Kaptan (2013). Since the TRIX revision was just based on observations in the oligotrophic Eastern Mediterranean, the revised TRIX scales in Table 4 are have very similar scale ranges to assess oligotrophic, mesotrophic, and eutrophic water bodies in the Eastern Mediterranean and Aegean Seas, but lower than the classical TRIX index ranges. The revised TRIX values calculated for the water bodies of the Mersin and Iskenderun Bays indicate development of mesotrophic/eutrophic conditions locally in these semi-enclosed water bodies (Fig. 2), due to NO_x rich river inflows with modified N/P/Si ratios and direct discharges of urban wastewaters (Dogan-Saglamtimur and Tugrul 2004; Tugrul et al. 2011).

The present results and previously scaling efforts (Primpas and Karydis 2011; Kaptan 2013) in the oligotrophic marine environments clearly show that the scaling ranges of classical TRIX index could not provide sufficient resolution for the assessment of eutrophic/mesotrophic trophic status of coastal and shelf waters in the NE Mediterranean and Aegean Seas having oligotrophic properties in the offshore. Apparent variability in the reference conditions of water masses on sub-basin scale has forced scientists to revise the ranges of TRIX scale based on systematic observations (Pettine et al. 2007; Primpas and Karydis 2011; Kaptan 2013). Moreover, it is

Table 5 Winter average values and averages of all data set of eutrophication-related parameters in surface waters of coastal and offshore areas of the Mersin Bay in 2008–2016 period

Inshore/winter	Salinity	TP (μM)	PO_4 (μM)	NO_x (μM)	DIN (μM)	DO (% sat.)	DWDO (% sat.)	Chl-*a* (μg/L)	SDD (m)	TRIX	E.I.
Mean	38.11	0.34	0.08	3.92	4.81	107.7	104.7	0.84	3.00	3.84	1.48
Std. dev.	0.71	0.16	0.04	3.62	4.20	7.4	8.5	0.55	1.27	1.00	1.21
N	142	119	152	162	161	161	100	124	91	123	161
Offshore/winter	Salinity	TP (μM)	PO_4 (μM)	NO_x (μM)	DIN (μM)	DO (% sat.)	DWDO (% sat.)	Chl-*a* (μg/L)	SDD (m)	TRIX	E.I.
Mean	39.21	0.13	0.03	0.32	0.51	100.1	99.2	0.19	15.15	1.69	0.18
Std. dev.	0.16	0.05	0.01	0.3	0.31	1.1	1.3	0.1	5.83	0.63	0.09
N	67	62	75	69	68	72	56	58	55	59	68
Inshore	Salinity	TP (μM)	PO_4 (μM)	NO_x (μM)	DIN (μM)	DO (% sat.)	DWDO (% sat.)	Chl-*a* (μg/L)	SDD (m)	TRIX	E.I.
Mean	38.61	0.29	0.05	1.43	2.04	108.8	103.1	0.67	4.3	3.83	0.66
Std. dev.	0.68	0.17	0.03	2.22	2.75	8.3	8.3	0.56	2.1	0.79	0.82
N	502	281	481	488	481	428	200	276	263	245	475
Offshore	Salinity	TP (μM)	PO_4 (μM)	NO_x (μM)	DIN (μM)	DO (% sat.)	DWDO (% sat.)	Chl-*a* (μg/L)	SDD (m)	TRIX	E.I.
Mean	39.31	0.14	0.03	0.17	0.38	101.6	99.6	0.12	15.4	1.79	0.15
Std. dev.	0.29	0.06	0.01	0.12	0.16	2.1	5.1	0.09	7.6	0.59	0.07
N	324	313	319	304	295	272	244	305	198	326	307

crucially necessary for the less productive Mediterranean sub-basin to obtain better classification of current trophic status after revision of the TRIX scaling ranges and validation of the classification results based on systematic data at sub-basin scale.

Eutrophication Index

Eutrophication Index (E.I.) is a multi-metric (combination of nutrients and chl-*a*) tool for the assessment of trophic status proposed by Primpas et al. (2010) for the Aegean coastal waters having oligotrophic properties in the offshore. The E.I. values calculated from the 2014–2016 data sets obtained in NE Mediterranean shelf (Fig. 1b) varied regionally and seasonally between 0.06 and 12.9 (Table 2). Spatio-temporal variability of E.I. is expectedly very consistent with those seen in the TRIX values (Fig. 2). However, only 20 E.I. values (out of 324 calculations; in Table 2) were higher than 0.85 and classified as "affected by eutrophication," and these coastal waters of the NE Mediterranean shelf and bays have "Poor" to "Bad" class of ecological quality according to the E.I. scale proposed by Primpas et al. (2010) (Table 4). The E.I. values calculated from the present nutrient and chl-*a* data sets suggest that the E.I. classification scale derived from the northern Aegean coastal bay data sets is not sensitive enough to differentiate the NE Mediterranean water bodies having "Good/Moderate" and "Moderate/Poor" classes of trophic status based on the modified TRIX scale and observations. Moreover, according the E.I. classification scale, 278 E.I. values (of 52 stations) out of 324 observations at 65 stations represent "Good" class of trophic status (Figs. 4 and 5); there is no "High" class level of water bodies, displaying reference conditions in the NE Mediterranean shelf areas though the salty offshore waters display oligotrophic properties with low nutrient and low chl-*a* concentrations (Yılmaz and Tugrul 1998; Kress and Herut 2001; Krom et al. 2004). It appears that the ranges of E.I. tool scale do not properly sort oligotrophic water bodies in the NE Mediterranean shelf because the weights of DIN (nitrate, ammonia, nitrite) coefficients used in the E.I. equation (footnote in Table 2) are much greater than the weight of reactive phosphate present at nearly detectable levels (0.02–0.05 μM) in the NE Mediterranean surface waters. The reduction of DIN weight in the E.I. calculations and replacing PO_4 by total phosphorus (TP) may improve the efficiency of E.I. tool for the oligotrophic NE Mediterranean waters. Since the NE Mediterranean shelf and open surface waters are highly depleted in PO_4, the N/P ratio is greater than the Redfield Ratio (16), leading to the development of P-limited algal production particularly in the coastal areas fed by DIN-laden terrestrial (natural+anthropogenic) inputs (Tufekci et al. 2013).

Chlorophyll-*a* (Chl-*a*)

Surface layer chl-*a* concentrations in the NE Mediterranean shelf waters displayed spatio-temporal variations between 0.02 and 1.75 μg/L for the 2014–2016 periods (Fig. 2; Table 2) as previously reported by Tugrul et al. (2011). Nutrients carried by the major rivers of Cilician Basin have led to enhancement of algal biomass (in terms of chl-*a* concentrations) in less saline coastal waters; the chl-*a* decreased to background levels (< 0.1 μg/L) in the offshore waters displaying open sea properties. Based on surface chl-*a* values, trophic status of the NE Mediterranean shelf waters was also classified by using the chl-*a* dependent 5-level classification scale proposed by Simboura et al. (2005). According to concentration ranges of Chl-*a* Index given in Table 4, the locations where 284 chl-*a* measurements in the surface layer (out of 323 observations at 65 stations) remained below 0.4 μg/L are classified to have "Good" to "High" level of trophic status (Figs. 4 and 5). However, the number of locations classified as "poor" levels of water quality according to the TRIX and E.I scales is greater than the hot spots (only 3 stations out of 65) characterized by chl-*a* scaling method to have "Poor" trophic status.

Scrutinization of the scaling and classification results in Tables 2 and 4 reveals that the 5-class scaling ranges of the classical TRIX, E.I., and chl-*a* indices for ecological water quality assessments need further revisions for the oligotrophic NE Mediterranean waters. Particularly, the "reference" and "good" quality boundary levels of these indices do not fully represent the "oligotrophic water body" occupying the NE Mediterranean shelf.

HELCOM Eutrophication Assessment Tool

HELCOM Eutrophication Assessment Tool (HEAT) was originally developed for the Baltic Sea to determine eutrophication status (HELCOM 2009; Andersen et al. 2011). The HEAT method, using site-specific reference conditions for each coastal water body (CWB), has been widely used in the Baltic Sea receiving large volumes of contaminated fresh water inputs and various types of wastewater discharges. This tool is principally based on the assessment of human-induced eutrophication status of different water bodies using their own "reference" conditions defined by expert groups for each water body having different ecological properties and trophic status. However, determination of the reference concentrations of eutrophication indicators for each water body needs thorough examination of the long-term data sets and expert judgment based on the surface salinity data and major terrestrial sources of inputs during the study period. Therefore, the sensitivity and reliability of HEAT method are higher than the other tools based on "single reference condition" for the entire system provided that "reference value" of each

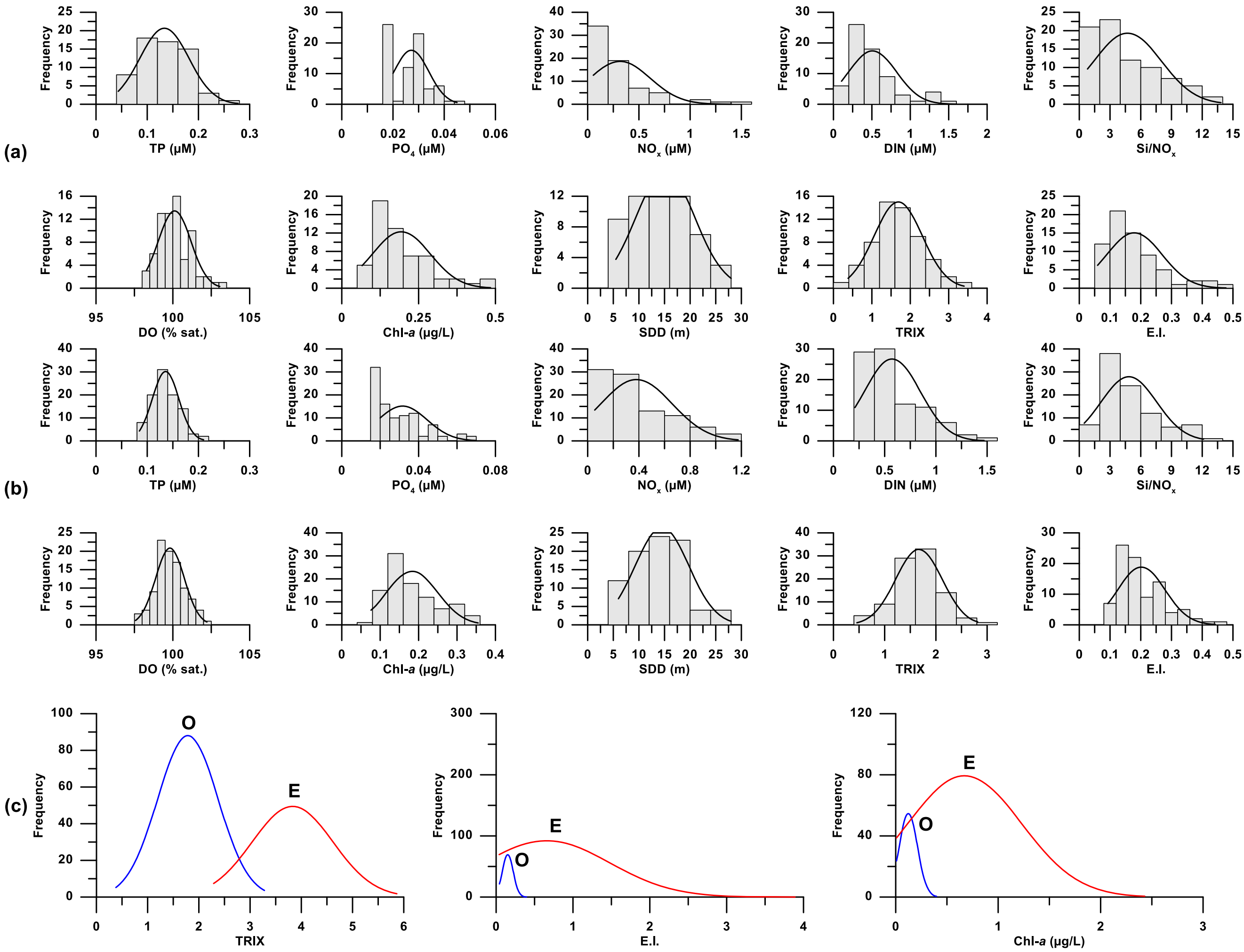

Fig. 3 Frequency distributions of **a** winter values of the eutrophication indicators in offshore waters of Mersin Bay in 2008–2016 period and **b** NE Mediterranean between 2014 and 2016 and **c** oligotrophic/eutrophic ranges of the eutrophication assessment tools; TRIX, E.I., chl-*a* scales

eutrophication parameter is determined properly using various approaches based on long-term data sets. Fortunately, it is possible for the Mediterranean Sea having similar reference conditions in the coastal and offshore waters. Based on the properly defined reference concentrations, the HEAT tool calculates Ecological Quality Ratio (EQR) values of the major eutrophication indicators for each water body studied and the final trophic status is determined by the "one out-all out" principle of integrated method (Andersen et al. 2011, 2015). In the Baltic Sea, the reference concentrations of nutrients

Table 6 Concentration ranges of eutrophication-related parameters for the Eastern Mediterranean Water Quality Classification by the HEAT method

Parameter	Highly eutrophic (bad)	Eutrophic (poor)	Mesotrophic (moderate)	Oligotrophic (good)	Reference condition*
TP (μM)	≥ 0.35	0.25–0.34	0.19–0.24	0.14–0.18	≤ 0.13
PO_4 (μM)	≥ 0.095	0.067–0.094	0.052–0.066	0.036–0.051	≤ 0.035
NO_x (μM)	≥ 1.08	0.77–1.07	0.60–0.76	0.41–0.59	≤ 0.40
DIN (μM)	≥ 1.62	1.15–1.61	0.90–1.14	0.61–0.89	≤ 0.60
Chl-*a* (μg/L)	≥ 0.49	0.35–0.48	0.27–0.34	0.19–0.26	≤ 0.18
SDD (m)	≤ 5	6–7	8–10	11–13	≥ 14

*Estimated from the average values of the winter data sets of 2008–2016 period in the NE Mediterranean shelf and bays

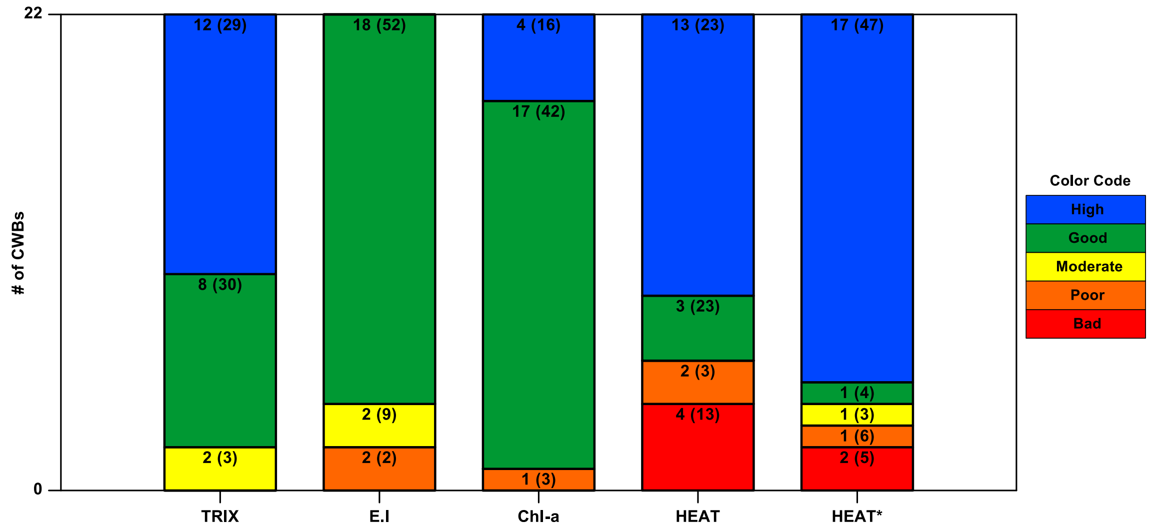

Fig. 4 Distributions of the eutrophication status of 22 coastal water bodies (values in parenthesis show # of stations) according to TRIX, E.I., chl-*a* values and HEAT method for the 2014–2016 period (5 surveys) (*HEAT: calculated by equal weights of EQRs for TP, PO_4, NO_x, and DIN)

were estimated from winter data sets which reflect maximum accumulation of inputs from atmosphere and land based sources. The reference values for algal biomass (chl-*a*) and water clarity (SDD) were derived from systematic data sets of high productive season (Andersen et al. 2011).

Reference values for the NE Mediterranean In order to apply HEAT tool for the classification of the NE Mediterranean shelf and bay water bodies (salinity > 38.5), we have determined a single "reference concentration" for each indicator based on the winter and summer data sets obtained by METU-IMS between 2008 and 2016 from the Mersin Bay and NE Mediterranean. For this goal, first, ranges of variations of eutrophication-related parameters were determined then statistical analysis of all the data was performed as described in Ignatiades et al. (1992). Second, ranges of surface concentrations and SDD data sets were separated as human-induced water body in the coastal zone (S < 39.0 and total depth ranging from 7 to 30–40 m) and offshore waters (S > 39.0; depth > 40 m) affected from terrestrial inputs at minimal levels. Then, each data set was evaluated after removing outliers according to method in Crawley (2007) and concentration ranges of eutrophication indicators for the coastal and offshore water bodies of the Mersin Bay were compiled in Table 5. Similarly, data from the least affected sites of NE Mediterranean coastal and open seas (Fig. 1b) were pooled and examined to determine their ranges, means, and standard deviations (Table 5) and frequency distributions of these parameters (Fig. 3). The frequency distributions of the eutrophication indicators and the calculated TRIX values in the Mersin Bay coastal and the offshore waters between 2008 and 2016 (Fig. 3) and from winter data of NE Mediterranean were used to estimate the "reference condition" values used for the assessment of eutrophication status of water bodies by the HEAT method. The frequency distributions illustrated in Fig. 3 were also used to determine the boundary values of oligotrophic/mesotrophic (unaffected (good)/affected (moderate) by eutrophication) and mesotrophic/eutrophic water bodies by the TRIX and HEAT tools (Fig. 3). The TRIX frequency distributions in Fig. 3 clearly demonstrate that the overlapping of oligotrophic and eutrophic water type data sets is pretty weak, indicating that the area within the overlapping zone (generally between 20- and 40-m depth zone) represent the mesotrophic water property in Mersin Bay.

The "reference condition" for each parameter was determined based on the frequency distributions of combined data sets in the inshore and offshore for summer and winter periods and their basin-scale surface layer (down to 10–20 m). The highest reference values in Table 6 were derived from the mean values of winter data sets (Table 5); the good/moderate, moderate/poor, and poor/bad class boundary values of nutrients and chl-*a* values were determined based on the HEAT approach (Andersen et al. 2011) and collated in Table 6, representing the "Reference Condition" and"Unaffected/Affected (good/moderate) boundary values for the entire NE Mediterranean water bodies. Based the boundary values in Table 6, the classification results of the summer-winter average values of 65 stations and 22 Coastal Water Bodies (CWBs) are summarized in Fig. 4.

Evaluation of data sets according to the classification scale ranges in Table 6 shows that trophic status of 99 observations (out of 324 observations) at 23 stations within NE Mediterranean shelf were unaffected by eutrophication and classified to have "High" level of trophic status. Only 13 stations (out of 65 stations) were affected by eutrophication and classified as "Bad" level water quality (Fig. 4). The present results clearly show that the HEAT tool can produces more sensitive and reliable results of the trophic status of the water

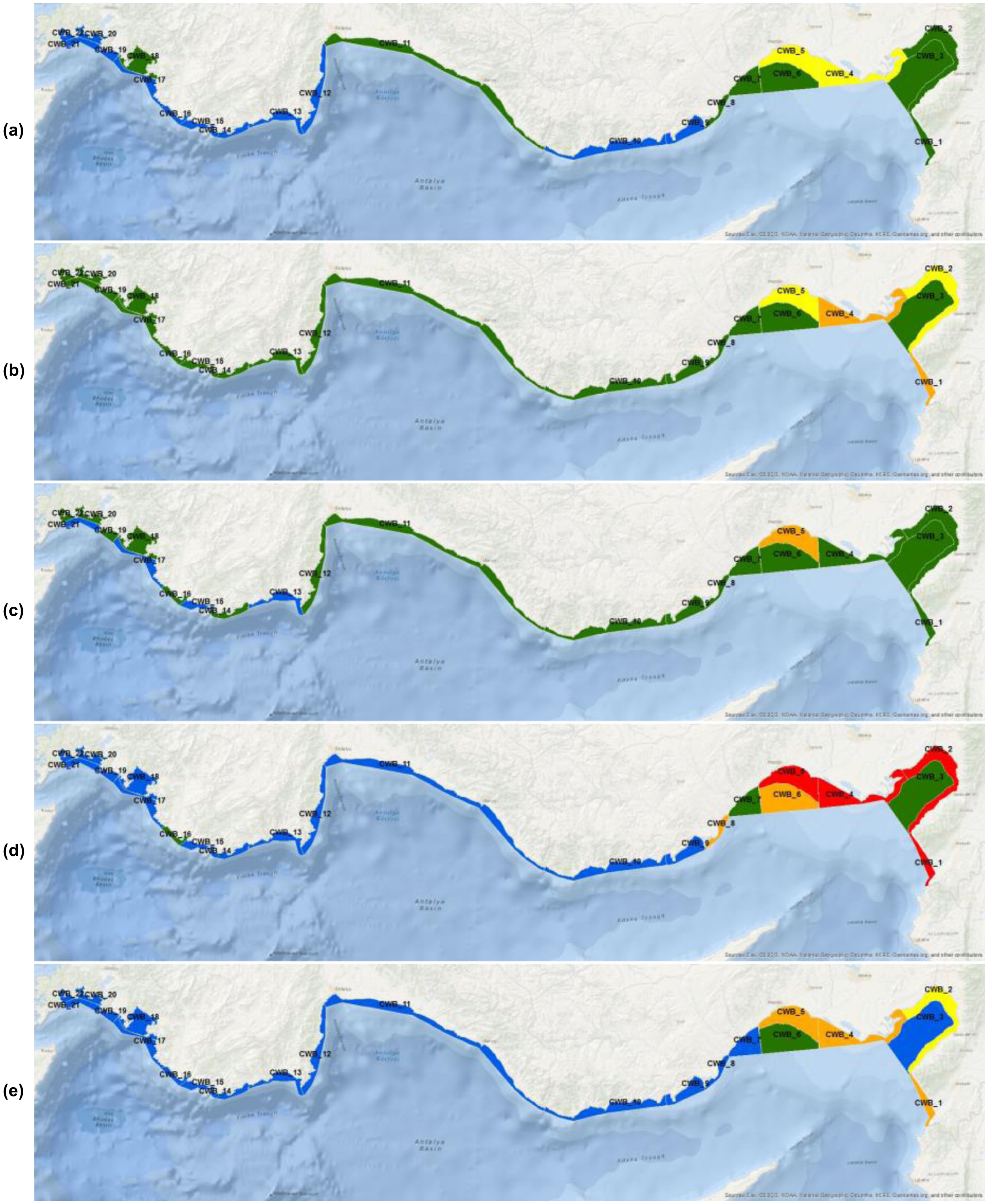

Fig. 5 Assessment of trophic status of coastal water bodies in the NE Mediterranean by the methods of **a** TRIX, **b** E.I., **c** Chl-*a*, **d** HEAT, and **e** HEAT by applying equal weights of EQRs for TP, PO_4, NO_x, and DIN values (color codes: red = bad, orange = poor, yellow = moderate-affected by eutrophication, green = good, and blue = high-unaffected by eutrophication) from the data obtained from winter-summer average values between 2014 and 2016

bodies influenced by eutrophication in the coastal and enclosed marine environments of NE Mediterranean having "single reference condition" value for each indicator as initially validated in the NE Mediterranean shelf waters ($S > 38.0$). When it is considered the one-out-all-out approach used in HEAT Index, individual stations would be easily classified as "poor or bad" with only one bad parameter though the rest of the parameters are outstanding. However, when water bodies, showing similar physical and biochemical features, are brought together to produce a "CWB" comprising more than one station occupying larger coastal area, the classifications of water bodies become more reliable. Based on typological and oceanographic properties of shelf and coastal regions, 22 CWBs were defined along NE Mediterranean from the Iskenderun to Marmaris (Turkey); the eutrophication classification of the CWBs was determined based on data sets obtained for each CWB and boundary conditions given in Table 6. The classification results of different tools are collated in Fig. 4 to compare their sensitivity and adequacy for the NE Mediterranean water bodies.

In the Eastern Mediterranean, "one out-all out" principle of the first version of HEAT tool (Andersen et al. 2011) is not adequate in the case of excess NO_x and P-depleted NE Mediterranean coastal regions where algal production mainly limited by reactive phosphorus. Boundary values estimated for NO_x forced the HEAT method to increase the number of locations having "poor" and "bad" class of water quality status though other parameters indicate "high" class ecological properties (unaffected by eutrophication). To overcome this challenge in determining acceptable nutrient level of CWB, the average of "observed value/reference condition ratio" value within an indicator category have been used to assess class value of each category and then "one out-all out" principle of the HEAT tool is followed for the classification of each CWB (Andersen et al. 2015). In this study, similar approach was applied to the nutrient data to determine to class level of the nutrient indicators and then the final eutrophication class of each CWB (Figs. 4 and 5).

Classification results of revised TRIX scaling ranges

The ranges of classical TRIX scale were revised for the NE Mediterranean coastal and bay water bodies, based on the frequency distribution of TRIX and eutrophication-related parameters illustrated in Fig. 3 for the Mersin Bay. Based on the revised ranges of TRIX index values in Table 4, average TRIX values of the NE Mediterranean water bodies were calculated and illustrated in Fig. 3 for the 2014–2016 period. Comparison of the index ranges proposed for different water types indicates that the Aegean Sea version of TRIX Index is in line with the range values proposed for NE Mediterranean at sub-basin level. This consistency strongly suggests that it might be used as a common TRIX scale for the Aegean and NE Mediterranean having oligotrophic properties in the offshore and dynamic coastal waters (UNEP 1989; Yılmaz and Tugrul 1998; Kress and Herut 2001; Krom et al. 2004; Tanhua et al. 2013 and references therein). According to TRIX and other scaling results (Figs. 4 and 5), the impact of major rivers on the trophic status of CWB's in the Cilician Basin is significant. Impacts of anthropogenic inputs, being significant at hot points (see Fig. 5), weaken in the CWBs and lead to assessment of higher ecological water quality due to dilution of inputs and removal by biochemical processes.

The classical TRIX tool does not provide sensitive resolution for the assessment of trophic status of coastal and shelf waters in the NE Mediterranean and Aegean Seas displaying oligotrophic properties in the offshore waters. New applicable approaches are still needed for the development of multimetric scaling tools to produce more efficient eutrophication assessment. The first step is to overcome the challenge of determination of a site-specific "reference condition" in the marine environments where eutrophic conditions developed. It allows us to determine sustainable "threshold values" for the implementation of sustainable marine policies and action plans in the marine environments having hydro-dynamically and biochemically different enclosed seas sensitive to human-induced pressures. In eutrophic coastal or enclosed seas, the assessment of "threshold values" can only be proposed by modeling studies as already experienced in the Baltic Sea. Higher threshold values estimated for NO_x forced the HEAT method to increase the number of locations having "poor" and "bad" water quality status though other eutrophication parameters indicate the presence of "high" level of water quality (Fig. 4). Therefore, the latest version of the HEAT tool uses the average or weighted average within an indicator category (nutrient levels, direct or indirect indicators) for "one out-all out" principle to assess the final class score of each CWB (Andersen et al. 2015) as proposed in the E.I. method of Primpas et al. (2010).

Conclusions

In the present study, different eutrophication classification tools (TRIX versions, E.I. and chl-*a* scaling, and HEAT) were utilized to determine current trophic status of the NE Mediterranean shelf waters and bays of Turkey. Their classification sensitivities were compared to understand their efficiency in the assessment of the actual eutrophication status of NE Mediterranean shelf/coastal waters. The classification results obtained by the classical TRIX are not in line with the result calculated from the revised TRIXs, HEAT, and E.I. scaling tools. The revised TRIX and E.I. tools are not capable of differentiating the actual eutrophication development if the coastal ecosystem has notable fresh water inflows spreading over large areas. If available data sets are sufficient for the reference condition assessment, the HEAT tool is capable of solving such challenges and flaws better than the others. Outcomes of this study are potential and can be used in future implementation of sustainable marine policies and action plans for the region.

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