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Identification of Type-Specific Phytoplankton Reference Conditions in Türkiye's Coastal Water Bodies

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Abstract Type-specific reference conditions for phytoplankton, along with models linked to relevant environmental factors, have been identified for Türkiye's coastal water bodies. Phytoplankton and water samples were seasonally collected (spring, summer, and fall) from 78 stations across 16 different water body types in the Aegean Sea, Mediterranean Sea, Black Sea, and Marmara Seas with in the REFCOND project of Türkiye between 2016 and 2020. Canonical Correspondence Analysis (CCA) was employed to identify the most significant environmental parameter associated with phytoplankton biomass, with total phosphorus (TP) emerging as the key parameter. The CCA indicated that the first axis accounted for a substantial proportion of the variance (72.26%), and ANOVA confirmed that only this axis was statistically significant. Phytoplankton biomass was deemed crucial for assessing the ecological status of coastal waters. Numerous statistically significant regressions linked phytoplankton biomass and chlorophyll-a (Chl-a, an indicator of phytoplankton biomass) to TP. For the first time, reference conditions and class boundaries for both phytoplankton biomass and Chl-a were established across all 16 water body types in Türkiye's coastal waters.

Keywords WFD · Reference conditions · Class boundaries · Phytoplankton · EQR · Chl-a

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1 Introduction

The Water Framework Directive (WFD) has been implemented since 2000 to restore and sustain good water status in all surface and groundwater bodies within the EU (Anon., 2000). The WFD provides an extensive legislative framework that focuses on water resource protection and the management of water usage.

The WFD mandates ongoing monitoring of various parameters, including physical, chemical, and biological factors, to assess water quality and ecological status (Bruin et al., 2005). These parameters are typically measured for both surface and groundwater, with the results used to identify pressures and impacts, set targets, and implement measures aimed at achieving and maintaining good status (Common Implementation Strategy for WFD 2003). Member

states are required to report on the status of water resources every six years.

As a key biological quality element under the WFD, phytoplankton react to nutrient enrichment, which can lead to eutrophication and harmful algal blooms. These blooms may cause hypoxia, anoxia, and other negative environmental effects. For instance, a recent massive bloom in the Marmara Sea resulted in significant mortality of benthic organisms and fish due to oxygen depletion (Maritime Executive 2021, May 26). Türkiye has embraced the WFD framework to manage its water resources under the leadership of the Ministry of Agriculture and Forestry (Erkan, 2014). A range of biomonitoring initiatives have been implemented, including biomonitoring projects conducted in river basins as part of the River Basin Management Plan (RBMP) preparation. Specific studies have been carried out to identify reference areas. The "Establishment of Reference Monitoring Network in Türkiye Project" (2016–2020) identified areas in 25 river basins and coastal-transitional waters that are free from anthropogenic pressures.

Under the Water Framework Directive (WFD), establishing a robust typology of surface water bodies is a prerequisite for defining appropriate reference conditions, as both are foundational for ecological status classification. Typology accounts for the natural variability of water bodies—such as altitude, depth, size, geology, and salinity—thereby enabling comparisons between water bodies of similar characteristics (System A and B; WFD Annex II). Reference conditions must be type-specific to ensure that assessments of ecological quality are based on deviations from natural or near-natural states relevant to each water body type (Hering et al., 2010). Agreeing on a consistent typology and corresponding reference conditions is essential to ensure that the ecological quality ratio (EQR) scale (van de Bund & Solimini, 2007). Consequently, type-specific reference conditions serve as the scientific benchmark against which anthropogenic impacts can be measured accurately, highlighting the crucial role of consistent typological frameworks in ecological assessment and water management.

Evaluation of phytoplankton as a critical biological quality component necessitates calculation of deviations from reference conditions to determine water quality. According to the WFD, there are four methodologies for establishing reference conditions: using spatially distributed data, employing predictive modelling, analysing historical or paleoreconstructive data, and relying on expert judgment (Heiskanen et. al., 2004). Numerous studies have applied these methods in various contexts. For example, spatially distributed data were effectively used in Danish coastal systems by Carstensen & Henriksen (2009). Predictive modelling approaches are exemplified in nutrient threshold studies that integrate indices of nutrient concentrations (Devlin et al., 2007). Additionally, expert judgment has been a key tool where data are limited, as demonstrated in the work of van de Bund and Solimini (2007), who synthesized expert input across European regions to estimate reference conditions for multiple BQEs, including phytoplankton. These diverse approaches highlight both the flexibility and the complexity of establishing robust ecological baselines within the WFD framework. In the absence of unimpacted sites, historical records or paleoecological approaches may be applied, and expert judgment becomes necessary when data are insufficient (Heiskanen et al., 2004; McNellie et al., 2020).

However, significant challenges have emerged in the designation of the reference conditions. In the determination of reference conditions, major shortcomings identified included the lack of separate assessments for different stressors, the failure to account for inter-annual variability, and insufficient harmonization of methodologies (Hering et al., 2010). There is a lack of consensus on the definition of reference conditions, as countries use different historical baselines and hold varying perceptions of background water quality, leading to inconsistencies even within the same region (Poikane et al., 2019). These shortcomings directly hinder progress toward achieving the WFD's primary objective of attaining good ecological status.

Under the EU Water Framework Directive (WFD), setting reference conditions for coastal marine water phytoplankton is essential for evaluating the ecological status of these waters (Devlin et al., 2007). The WFD seeks to attain "good ecological status" by contrasting current conditions with reference conditions that reflect an undisturbed state (Birk et al., 2012). This process encompasses the assessment of phytoplankton composition, abundance, biomass, and seasonal succession. This study seeks to examine and define reference conditions and classification

boundary values to ensure an accurate ecological quality assessment for monitoring coastal marine waters. As a first step in determining type-specific reference conditions, the methodology based on the evaluation of existing monitoring data is intended to be applied for the first time in Türkiye.

2 Materials and Methods

2.1 Study Area

Türkiye's total coastline extends to 8.333 km, and it is bordered by the Black Sea, the Marmara Sea, the Aegean Sea, and the Mediterranean Sea. Seasonal surveys were conducted at 78 sampling stations along the Turkish coasts of the Aegean Sea, Black Sea, Marmara Sea, and Mediterranean Sea, in the months of April, July, and October (Fig. 1). The coordinates and types for these sampling stations are detailed in Table 1.

2.2 Sampling

Phytoplankton samples were collected both qualitatively and quantitatively using a plankton net with a 20 µm mesh size and a Hydrobios sampling bottle with a capacity 5 L. Sampling occurred at three different depths: 0.5 m, mid-depth, and near the bottom of the water column. Over the course of three years, a total of 78 sampling stations along the Turkish coastal waters were surveyed through 9 separate sampling campaigns conducted in three seasons annually (spring, summer and fall). The criteria for selecting reference sampling stations included their distance from densely populated areas, the absence of agricultural, industrial, and urban discharge sites, and a preference for natural or minimally modified coastlines (Heiskanen et al., 2004).

Phytoplankton analyses were conducted on samples preserved with Lugol's solution, following the Utermöhl method (Sournia, 1978). The cells were enumerated using a Sedgwick-Rafter counting chamber, and taxonomic classification adhered to the

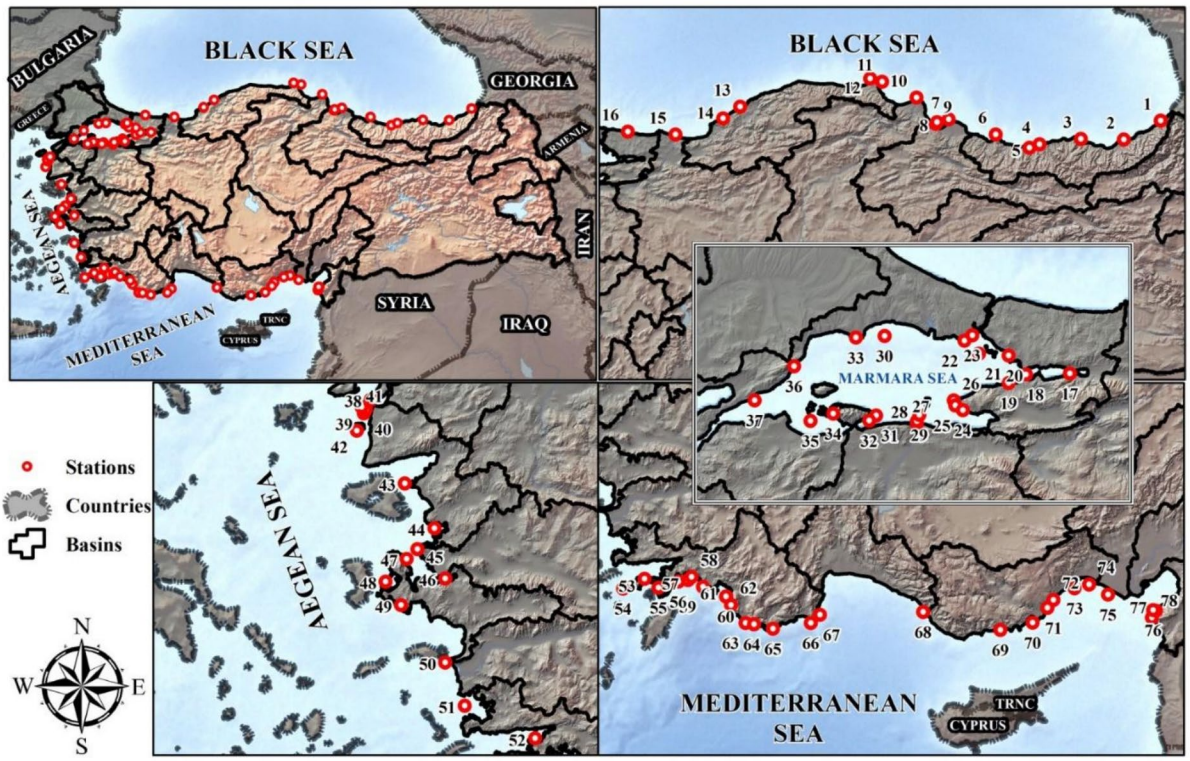

Fig. 1 The positions of the sampling stations

Table 1 The coordinates and types of sampling stations. S: Salinity‰)), D: Depth. Type specifications were obtained from DGEM (2014)

SEA	CODE	TYPE	TYPE SPECIFICATIONS	LATITUDE	LONGITUDE
BLACK SEA	1	KD-7	S < 17.5, D > 30 m, Sedimentary bottom	41.37246	41.31227
	2	KD-7		41.05901	40.49884
	3	KD-7		41.06996	39.56015
	4	KD-4	S > 17.5, Depth < 30 m, Hard bottom	40.98160	38.64552
	5	KD-7		40.92432	38.42612
	6	KD-4		41.14580	37.67982
	7	KD-5	S < 17.5, Depth < 30 m, Sedimentary bottom	41.39349	36.65018
	8	KD-4		41.34508	36.38990
	9	KD-3	S > 17.5, Depth < 30 m, Sedimentary bottom	41.31993	36.36322
	10	KD-7		41.75244	35.94027
	11	KD-3		42.01332	35.18330
	12	KD-3		42.06059	34.91665
	13	KD-7		41.60139	32.05294
	14	KD-5		41.40941	31.68087
	15	KD-5		41.14357	30.62932
	16	KD-5		41.19281	29.58677
MARMARA SEA	17	MD-2	Depth < 15 m, Sedimentary bottom	40.72999	29.78269
	18	MD-6	Depth > 30 m, Sedimentary bottom	40.72347	29.39972
	19	MD-6		40.66738	29.24828
	20	MD-6		40.85018	29.24694
	21	MD-6		40.86253	28.98827
	22	MD-4	15 m < Depth < 30 m, Sedimentary bottom	40.94706	28.85942
	23	MD-4		40.98358	28.93065
	24	MD-6		40.48628	28.84707
	25	MD-2		40.51907	28.77947
	26	MD-6		40.55154	28.76519
	27	MD-2		40.39944	28.44174
	28	MD-4		40.41420	28.44353
	29	MD-6		40.45611	28.46449
	30	MD-6		40.97959	28.16152
	31	MD-1	Depth < 15 m, Hard bottom	40.45179	28.08670
	32	MD-6		40.41647	28.02432
	33	MD-1		40.96759	27.91048
	34	MD-1		40.46312	27.71152
	35	MD-6		40.41560	27.51264
	36	MD-6		40.77832	27.36888
	37	MD-6		40.55140	27.02125
AEGEAN SEA	38	AE-3	S < 34.5, Depth < 40 m, Sedimentary bottom	40.06069	26.15978
	39	AE-1	S < 34.5, D > 40 m, Sedimentary bottom	39.99290	26.05230
	40	AE-3		39.97316	26.09942

Table 1 (continued)

SEA	CODE	TYPE	TYPE SPECIFICATIONS	LATITUDE	LONGITUDE
	41	AE-4	S < 34.5, D < 40 m, Hard bottom	39.93721	26.07878
	42	AE-9	S > 37.5, D > 40 m, Sedimentary bottom	39.78039	25.99683
	43	AE-11	S > 37.5, D < 40 m, Sedimentary bottom	39.30054	26.56244
	44	AE-10	S > 37.5, D < 40 m, Hard bottom	38.88991	26.90960
	45	AE-10		38.69773	26.70819
	46	AE-7	34.5 < S < 37.5, D < 40 m, Sedimentary bottom	38.42826	27.03674
	47	AE-9		38.60931	26.58296
	48	AE-9		38.39869	26.33396
	49	AE-11		38.18120	26.51749
	50	AE-10		37.64825	27.03783
	51	AE-9		37.24329	27.26993
	52	AE-11		36.93632	28.09684
MEDITERRANEAN SEA	53	AE-9		36.79180	27.75097
	54	AE-10		36.66550	27.41145
	55	AE-11		36.67757	27.97077
	56	AE-9		36.75633	28.28390
	57	AE-10		36.77498	28.43708
	58	AE-11		36.81356	28.49555
	59	AE-9		36.69121	28.67332
	60	AE-8	S > 37.5, D > 40 m, Hard bottom	36.56782	29.04054
	61	AE-10		36.56576	29.04470
	62	AE-11		36.45893	29.11936
	63	AE-9		36.22749	29.34595
	64	AE-10		36.21235	29.48204
	65	AE-11		36.15722	29.78025
	66	AE-9		36.22928	30.37949
	67	AE-10		36.33507	30.52909
	68	AE-9		36.36955	32.16033
	69	AE-10		36.13959	33.38607
	70	AE-11		36.23355	33.89467
	71	AE-9		36.42109	34.12628
	72	AE-10		36.52411	34.22180
	73	AE-9		36.67183	34.55556
	74	AE-11		36.72010	34.78801
	75	AE-11		36.59080	35.08981
	76	AE-9		36.39257	35.80330
	77	AE-11		36.38633	35.84458
	78	AE-10		36.29865	35.78087

scheme outlined by Lee (1999). Lugol-fixed water samples were concentrated to volumes ranging from 1 to 3 ml and subsequently examined under an inverted microscope using the Sedgewick-Rafter chamber (Guillard, 1978). Phytoplankton species were categorized according to their taxonomic groups. Identification of phytoplankton taxa was performed using the following keys: Round et al. (1990), Krammer and Lange-Bertalot (1991a, b; 1999a, b), Sims (1996), Hasle and Syvertsen (1997), Steidinger and Tangen (1997), Throndsen (1997), Lange-Bertalot (2000), John et al. (2003), and Hallegraeff (2004). The biovolume of each taxon was calculated by multiplying the cell density by the unitary cell biovolume, which was determined through geometrical approximations (Hillebrand et al., 1999). Biovolumes were then converted to biomass according to the study of Menden-Deuer and Lessard (2000).

2.3 Environmental Parameters

Secchi depth (SD, m), temperature (*T*, ºC), pH, dissolved oxygen (DO, mg/L), salinity (S, ‰) were recorded directly on site. The water samples were then placed in iceboxes and quickly transported to the laboratory for subsequent analysis. Measurements of total organic carbon (TOC, mg/L), total nitrogen (TN, mg/L), dissolved inorganic nitrogen (DIN, mg/L), total phosphorus (TP, mg/L), alkalinity (ALK, mg/L CaCO3), silica (SI, mg/L), and chlorophyll-a (Chl-a, µg/L) were conducted following the methods outlined in APHA (2012).

2.4 Statistical Analysis

All statistical analyses and graphical visualizations were conducted using R software (version 4.3.1, R Core Team, 2021). To mitigate skewness, environmental variables were log-transformed (Borcard et al., 2018). A linear regression analysis was conducted to explore the relationship between Chl-a and TP (key environmental parameter), aiming to link phytoplankton with potential eutrophication indicators. Initially, the Chl-a and TP data were prepared. Outliers were addressed using two methods: first, outliers were removed based on whiskers criteria, and then an R package for outlier detection was employed (Komsta and Novomestky, 2022, 2015). To minimize noise, an average was computed using the highest values, median, and half of the detection limit for Chl-a, aligned with total phosphorus values. The normality of the residuals was assessed using the Jarque–Bera test (Jarque & Bera, 1987).

To determine the appropriate ordination method for analysing biomass data, we adhered to the guideline proposed by Lepš and Šmilauer (2003). Initially, we performed a Detrended Correspondence Analysis (DCA) on our dataset and assessed the length of the first DCA axis, which is measured in standard deviation units. The length of this axis was 4.04, exceeding the threshold of 4 standard deviations, suggesting that our dataset is heterogeneous and thus suitable for unimodal ordination methods. For comparison with the results of a linear regression model applied to Chl-a data and TP data, we conducted a CCA on species biomass data transformed using the Hellinger transformation. We excluded species contributing less than 1% to the total biomass to minimize analytical noise, following the recommendations of Legendre and Gallagher (2001). The vegan package was employed for the Canonical Correspondence Analysis (CCA) to identify the prevailing environmental parameters at the sampling stations (Oksanen et al., 2017). To define centroid groups, a custom function called Ordicenter was used within the vegan package. ANOVA was conducted to assess the significance of the CCA axes. To select relevant parameters, an initial tbPCA (two-block Principal Component Analysis) was performed, and regressions were calculated between the PCA axes and environmental parameters, with p-values adjusted using the Bonferroni correction (Legendre and Legendre, 1998). This approach allowed for the identification of key variables through unconstrained analysis.

2.5 The Process of Defining Type-Specific Reference Conditions and Establishing Class Boundary Values Ecological Quality Ratios (EQR)

The coastal waters of Türkiye were categorized into 25 distinct types based on criteria including region, depth, salinity, and substratum (DGEM, 2014). This study includes 16 of these types. The WFD mandates that ecological status be assessed for the classification of all European surface waters. This assessment is quantified using the Ecological Quality Ratio (EQR), which ranges from 1 (indicating high ecological status) to 0 (indicating bad ecological status). The EQR

is calculated by dividing the current value by the reference value. Reference conditions for the biomass were determined based on sites identified as reference according to the REFCOND procedure (REFCOND, 2003). The reference condition for biomass was defined as the 20th percentile of the biomass values from these reference sites. The boundaries for the ecological quality classes-High/Good (H/G), Good/Moderate (G/M), Moderate/Poor (M/P)-were set at the 40th, 60th, and 80th percentiles of the biomass data, respectively. For Chl-a reference conditions, the 90th percentile method was employed, with the 10th percentile (lower quartile) selected as the reference value based on consensus among the Geographical Intercalibration Groups (GIGs) (Carletti & Heiskanen, 2009). Class boundaries for Chl-a were established using the 10th, 20th, 40th, 60th, and 80th percentiles derived from Chl-a dataset.

3 Results

The evaluation of environmental parameters across various sample types demonstrated considerable variation in the recorded measurements (Table 2). The highest temperatures were noted in sample types AE-9 and KD-5, with recorded values of 26.9 °C and 27.4 °C in summer, respectively. Conversely, the lowest temperatures were observed in KD-3 and MD-1, at 15.8 °C in spring and 16.5 °C in fall. The pH levels varied, with the highest recorded at 8.74 in MD-1 and the lowest at 7.6 in KD-3 in the spring season. Alkalinity was most elevated in MD-1 and KD-7, measuring 117 mg/L and 114 mg/L in autumn, while the lowest values were found in KD-3 and KD-5, at 76 mg/L in fall.

Salinity peaked in AE-9 and AE-10 at 42 in summer, while the lowest values were recorded in KD-3 and KD-7, both at 14 in spring. Dissolved oxygen levels were highest in MD-1 at 11.6 mg/L in spring and lowest in KD-3 at 7.59 mg/L in summer. The total organic carbon concentrations were highest in KD-3 and KD-7, recorded at 4.73 mg/L and 5.69 mg/L in spring, respectively, while the lowest levels were detected in MD-1 and KD-3, at 0.1 mg/L in spring. Total nitrogen exhibited peak values in KD-3 (0.475 mg/L) in spring and KD-7 (0.614 mg/L), in summer with the minimum concentrations observed in KD-3 and KD-5, both at 0.1 mg/L in summer.

Dissolved inorganic nitrogen (DIN) concentrations were highest in KD-3 and KD-7, both at 0.297 mg/L in spring, while the lowest levels were recorded in KD-5 and KD-3, at 0.002 mg/L in summer.

Elevated TP concentrations observed in AE-8 and AE-9 during summer (0.008 mg/L) and in KD-5 within the Black Seasuggest localized nutrient enrichment, likely driven by the terrestrial or riverine inputs, thereby heightening the potential for eutrophication. Conversely, AE-1 and AE-8 display high Secchi depths and low TP levels (0.005 mg/L in spring), indicative of oligotrophic, high-transparency conditions charasteristic of offshore Aegean and Mediterranean waters. These spatial contrasts highlight the necessity for targeted nutrient management in specific nearshore types, while preserving the naturally low-nutrient status of in offshore regions.

Chl-a concentrations, which serve as an indicator of phytoplankton biomass, varied from 0.5 µg/L to 4.2 µg/L in the Mediterranean Sea, with the lowest levels observed at stations AE-1 and AE-8 in spring and the highest at station AE-7 in fall. In contrast, Chl-a concentrations reached their peak in KD-5 and KD-7 at 104 µg/L.

Figure 2 illustrates a positive yet moderate correlation between TP and Chl-a ($R^2 = 0.4056$), suggesting that although phosphorus constitutes an important driver of phytoplankton biomass, additional factors-such as light availability, hydrodynamic conditions, and grazing pressure-also exert significant influence. From a management standpoint, this implies that phosphorus reduction alone may be insufficient to curb biomass proliferation, underscoring the need for integrated monitoring that addresses multiple stressors. Generally, the Black Sea and Marmara types (KD-3, KD-4, KD-5, KD-7, MD-1, MD-2, MD-4, MD-6) exhibited elevated TP and Chl-a concentrations, whereas the Aegean and Mediterranean types-particularly AE-1, AE-3, AE-4, AE-7, AE-8-displayedoligotrophic characteristics. These patterns reflect the high nutrient loads in the Black Sea and Marmara Sea, attributable to substantial freshwater inflows combined with limited water exchange.

In total, 301 phytoplankton species from 12 different phyla were identified. The species that were dominant in biomass included *Achnanthes brevipes* C. Agardh, *Chaetoceros affinis* Lauder, *Dactyliosolen fragilissimus* (Bergon) Hasle in Hasle and Syvertsen, *Pseudo-nitzschia pungens* (Grunow ex Cleve) Hasle,

Proboscia alata (Brightwell) Sundström and *Thalassiosira gravida* Cleve from Bacillariophyta; *Scenedesmus ellipticus* Corda from Chlorophyta; *Teleaulax acuta* (Butcher) D.R.A.Hill from Cryptophyta; *Aphanizomenon flosaquae* Ralfs ex Bornet and Flahault, *Merismopedia elegans* A.Braun ex Kützing, *Phormidium limosum* (Dillwyn) P.C.Silva from Cyanobacteria; *Gymnodinium catenatum* H.W.Graham, *Lessardia elongata* Saldarriaga and F.J.R.Taylor, *Noctiluca scintillans* (Macartney) Kofoid and Swezy, *Prorocentrum micans* Ehrenberg, *Scrippsiella acuminata* (Ehrenberg) Kretschmann, Elbrächter, Zinssmeister, S.Soehner, Kirsch, Kusber and Gottsshelling, *Tripos eugrammus* (Ehrenberg) F.Gómez from Dinoflagellata, and *Phaeocystis globosa* Scherffel from Haptophyta. A one-way ANOVA was conducted to evaluate seasonal variations in phytoplankton biomass across the coastal water bodies. The analysis indicated a statistically significant effect of season ($F = 3.89$, $p = 0.0221$), highlighting the strong influence of seasonal dynamics on biomass levels. Phytoplankton biomass increased notably in spring (Fig. 3), with the Black Sea and Marmara Sea exhibiting significantly higher mean values compared to summer and fall. In contrast, seasonal variability was less pronounced in the Mediterranean and Aegean types, although localized anomalies-such as the fall Chl-a peak recorded in AE-7- were evident.

A CCA analysis (Fig. 4) was performed on biomass data, constrained by log-transformed variables (T, pH, S, TN, SD and TP). The first canonical axis (CCA1) accounts for a significant proportion of the variance among the constrained axes (72.26%), indicating the influence of eutrophication. The second axis (CCA2) explains 17.06% of the variance, reflecting the effects of temperature and the salinization (Fig. 4a-b). According to the ANOVA for axes, only CCA1 shows significant results (p-value = 0.036), while other axes (CCA2 to CCA6) are not significant (p-value > 0.05), indicating they do not capture substantial additional variation. Regression analysis reveals a significant relationship between CCA1 and TP (Fig. 5), with a positive TP coefficient (80.566) and statistical significance (p-value < 0.05), suggesting that increases in TP correspond to increases in CCA1. The model explains approximately 58.53% of the variance in CCA1 (R-squared = 0.5853) and is highly significant overall (F-statistic: 88, p-value < 0.05). Residuals show a reasonable distribution, with most values clustered around the median. Consequently, the samples formed two primary groups along CCA1. When the samples were categorized by type and repositioned based on the geometric mean center (using the ordicenter function from the vegan package), a typological separation emerged according to phytoplankton biomass (Fig. 4b). This typology proved meaningful for coastal water bodies in relation to phytoplankton. The first canonical axis (CCA1) primarily represented a eutrophication gradient, with strong positive loadings for TP and phytoplankton biomass. High-TP/high-biomass types such as KD-7, KD-5, and MD-6 were positioned at the positive end of CCA1, whereas oligotrophic types from the Aegean and Mediterranean Seas (e.g., AE-1, AE-4) occupied the negative end. The second canonical axis (CCA2) reflected a combined temperature–salinity gradient, distinguishing warmer, high-salinity Mediterranean types. For example, AE-9 and AE-10 were associated with higher salinity, whereas AE-1 and AE-4 clustered with higher transparency (higher SD values). Species contributing most strongly to these gradients included Pseudo-nitzschia pungens and Proboscia alata on the eutrophic side of CCA1, and Chaetoceros affinis and Thalassiosira gravida on the oligotrophic side. This separation indicates that CCA captured both nutrient-driven and hydrographic structuring of phytoplankton communities.

The first canonical axis (CCA1) accounts for a substantial portion of the variance (72.26%) among the constrained axes, reflecting the influence of eutrophication. The second canonical axis (CCA2) explains 17.06% of the variance, relating to temperature and the salinization. ANOVA results indicate that only CCA1 is statistically significant (p-value = 0.036). A) The samples grouped by sea basins, represented as colored dots, with blue arrows indicating the direction and impact of the environmental factors on the CCA ordination. B) The samples grouped according to the typology and centered based on the geometric mean of the types.

The 10 percentile (lower quartile) of the Chl-a dataset was used as a reference value. Boundary class values between H/G and G/M were established at the 20th and 40th percentiles, respectively. The boundaries for M/P and P/B boundaries were set at the 60th and 80th percentiles, respectively

(Table 3). The calculated Chl-a concentrations for the 10th to 80th percentiles, which define class boundaries for all water body types, ranged from 0.01 to 4.66 µg/L. For AE-1, AE-3, AE-4, and AE-7, the Chl-a values ranged from 0.34 (reference value) at the 10th percentile to 0.50 (Bad) at the 80th percentile. AE-8 and AE-9, values increased from 0.25 (reference value) to 0.63 (Bad). AE-10 values ranged from 0.53 (reference value) to 0.77 (Bad), while AE-11 ranged from 0.19 (reference value) to 0.63 (Bad). For KD-3, values rose from 0.53 (reference value) to 0.87 (Bad), for KD-4, they ranged from 0.32 (reference value) to 0.83 (Bad). KD-5 values increased from 0.56 (reference value) to 1.27 (Bad), and KD-7 showed an increase from 0.39 (reference value) to 1.01 (Bad). MD-1 and MD-2 values ranged from 0.62 (reference value) to 1.36 (Bad), MD-4 increased from 0.50 (reference value) to 1.01 (Bad), and MD-6 exhibited the highest increase, ranging from 0.52 (reference value) to 1.51 (Bad). These results highlight significant variability and dynamic changes in Chl-a concentrations across different water body types.

Reference conditions for phytoplankton biomass data were assessed for each water type (Table 4). The highest biomass was recorded at station KD-7, with a value of 1369 µg/L, indicating a high abundance of phytoplankton in this area. Conversely, the lowest biomass value was observed at stations AE-7, AE-4, AE-1 and AE-3, with 43 µg/L, suggesting a lower phytoplankton density in these regions. Station KD-7 also had the highest reference condition value of 274, while the lowest reference value was 9 at station AE-7, AE-4, and AE-3. The highest H/G value was 547 at station KD-7, whereas the lowest H/G value was 17 at AE-7, AE-4, and AE-3. Station KD-7 also exhibited the highest G/M value of 821, with AE-7, AE-4, and AE-3 showing the lowest G/M value of 26. The highest M/P value was 1095 at KD-7, while AE-7, AE-4, AE-1, and, AE-3 and AE-1 had the lowest M/P value of 35.

4 Discussion

Significant variability in environmental parameters has been observed across different sample types, reflecting highlighting the diverse ecological conditions along the Turkish coasts. These findings enhance the understanding of nutrient dynamics and their effects on phytoplankton biomass, which is crucial for establishing reference conditions and assessing ecological quality ratios.

Temperature (*T*) plays a critical role in influencing phytoplankton, affecting their metabolic rates, growth, community composition, and bloom timing, as well as impacting surrounding water properties such as isopycnal characteristics, dissolved gases, and solubility of solids (Behrenfeld et al., 2006). *T* observations in Türkiye are consistent with Mediterranean Sea temperature averages (24 °C-28°C) in summer, while lower temperatures align more closely with those of the Baltic and North Seas in spring and fall (16 °C-20°C and 15 °C-18°C, respectively) (European Environment Agency, 2023). The pH of water significantly affects phytoplankton physiology, growth rates, and species composition (Rost et al., 2006). Factors influencing pH include CO_2 absorption, biological activity, and freshwaters inputs (Omsted et al., 2010). The pH measurement of 8.74 in MD-1 (Marmara Sea) in spring suggests a highly alkaline environment, potentially indicative of a micro-environment within the Mediterranean (average pH 8.1–8.4) or Aegean Sea (average pH 8.2–8.5), where photosynthesis is notably active. Conversely, the pH measurement of 7.6 in KD-3 (Black Sea) in spring reflects an area with greater freshwater influence and organic activity, similar to the conditions found in the Baltic Sea (EMODnet, 2023).

Alkalinity, which indicates a water body's capacity to neutralize acids, affects phytoplankton by stabilizing pH, influencing nutrient availability, and altering overall water chemistry. Variations in alkalinity can shift phytoplankton community composition, favoring species that are adapted to specific pH and nutrient conditions (Reynolds, 2006). The high alkalinity observed in MD-1 (Marmara Sea) is consistent with the Mediterranean Sea, where high evaporation rates and carbonate concentrations contribute to elevated alkalinity. In contrast, the lower alkalinity measurements in KD-3 and KD-5 (Black Sea) align with regions impacted by substantial freshwater inputs, such as the Baltic Sea (Liu et al., 2010).

Salinity impacts marine phytoplankton in various ways, including their distribution, physiology, community structure, and nutrient uptake in marine environments (Telesh et al., 2013). The salinity

measurement for AE-10 (Aegean Sea) in summer suggests a region with high salinity and evaporation, characteristics of the Mediterranean or Aegean Sea. Conversely, the measurement for KD-7 (Black Sea) in spring indicates an area with lower salinity, likely influenced by freshwater inputs similar to those affecting the Black Sea or Baltic Sea (Copernicus Marine Service, 2023).

Dissolved oxygen (DO) levels are essential for assessing the health and productivity of phytoplankton populations in marine environments (Falkowski & Raven, 2007). Both high and low DO levels can influence phytoplankton photosynthesis, respiration, community composition and nutrient cycling (Diaz & Rosenberg, 2008). The high DO measurement of 11.6 mg/L in MD-1 (Marmara Sea) in spring suggests it may originate from a well-mixed, cooler region, similar to the Baltic Sea, where active photosynthesis occurs (Carstensen et al., 2005). In contrast, the DO measurement of 7.59 mg/L in KD-3 (Black Sea) in summer does not align with typical conditions of the Black Sea, which can experience fluctuations due to complex vertical mixing, upwelling and stratification events (Oğuz and Velikova, 2010).

Total Organic Carbon (TOC) plays a crucial role in phytoplankton growth, community composition, and overall ecosystem dynamics as a nutrient source (Wang et al., 2023). Elevated TOC levels can increase water turbidity, which diminishes light availability and restricts photosynthesis (Liu et al., 2021). High TOC levels observed in KD-3 and KD-7 (Black Sea) in spring are characteristics of regions such as the Baltic Sea or Black Sea, where high biological productivity and substantial riverine inputs contribute to elevated organic carbon concentrations (Oğuz and Velikova, 2010). Conversely, the low TOC Levels in MD-1 (Marmara Sea) in spring suggests areas with relatively low organic matter, typical of open waters in the Mediterranean or Aegean Seas (Giani et al., 2012).

Total nitrogen (TN) is vital for phytoplankton growth, influencing productivity, community composition, and overall ecosystem health due to its role in protein synthesis and cell development (Moschonas et al., 2017). Elevated TN levels observed in KD-3 and KD-7 (Black Sea) in spring are indicative of areas such as the North Sea or Black Sea, where nutrient inputs from rivers and anthropogenic activities lead to increased nitrogen concentrations (Ludwig et al., 2009).

DIN availability significantly enhances phytoplankton growth and biomass, particularly in nitrogen-limited environments. Excessive DIN can lead to algal blooms (Reusch et al., 2018). DIN comprises ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-). Ammonium is often the preferred nitrogen source for many phytoplankton species due to its direct uptake and assimilation, which promotes faster growth and higher productivity compared to other forms of nitrogen. However, nitrate (NO_3^-) and nitrite (NO_2^-) are also important nitrogen sources (Gruber, 2008). High levels of DIN observed in MD-6 (Marmara Sea) and KD-5 (Black Sea) in spring suggest areas with substantial anthropogenic nutrient inputs, potentially from urban or agricultural sources (Grizzetti et al., 2012).

Total phosphorus (TP) is crucial for ATP synthesis, nucleic acids, and cellular membranes, supporting phytoplankton growth and productivity, particularly in phosphate-limited environments (Wang et al., 2021). Excessive TP can result in harmful algal blooms, significantly increasing Chl-a levels and leading to oxygen depletion and biodiversity loss (Carstensen & Henriksen, 2009). The low TP levels observed in AE-4 and AE-8 in spring (Mediterranean Sea) reflect regions with low nutrient availability, characteristics of the oligotrophic Mediterranean, where phosphorus often acts as a limiting nutrient (Varkitzi et al., 2020).

Silica (SiO_2) plays a critical role in the growth and productivity of certain phytoplankton, particularly diatoms, which require silica for the formation of their cell walls (frustules). This essential nutrient directly impacts their ecological function within marine environments (Tréguer & De La Rocha, 2013). High concentrations of silica, such as those observed in KD-7 and KD-5 (Black Sea) in spring, are uncommon in open ocean environments and are more typical of regions near river mouths or coastal areas with silicate-rich geology, similar to the Baltic Sea. Elevated silicate levels in these areas are often attributed to substantial riverine inputs from catchments containing silicate-bearing minerals (Schneider et al., 2019).

The Secchi disk depth (SD) provides an important linkage for the monitoring program for eutrophication in marine waters—those with elevated nutrient supply rates of both nitrogen and phosphorus stimulate phytoplankton growth and reduce the clarity of water (Golubkov & Golubkov, 2024). In

Table 2 Averages of the environmental parameters across various sample types demonstrated considerable variation in the recorded measurements

TYPE	TP	CHL	T	DO	S	TOC	TN	ALK	SI	DIN	SD	PH
AE-1	0.007 ± 0.003	0.500 ± 0.000	22.333 ± 1.986	8.350 ± 1.645	34.400 ± 1.587	1.000 ± 0.000	0.626 ± 0.342	159.667 ± 4.041	0.179 ± 0.275	0.125 ± 0.110	11.330 ± 2.517	8.155 ± 0.166
AE-3	0.006 ± 0.002	0.963 ± 1.225	21.450 ± 2.921	8.990 ± 0.461	34.650 ± 4.606	1.000 ± 0.000	0.436 ± 0.280	152.167 ± 11.583	0.634 ± 1.290	0.073 ± 0.090	10.467 ± 4.378	8.255 ± 0.196
AE-4	0.005 ± 0.000	2.900 ± 3.394	22.533 ± 1.888	8.310 ± 1.146	29.867 ± 0.808	13.333 ± 21.362	0.504 ± 0.216	151.333 ± 6.110	0.111 ± 0.157	0.125 ± 0.110	0.500 ± 0.000	8.117 ± 0.350
AE-7	0.007 ± 0.003	4.200 ± 3.989	23.133 ± 2.802	8.357 ± 0.791	39.367 ± 4.477	1.000 ± 0.000	0.631 ± 0.113	149.200 ± 10.577	0.020 ± 0.000	0.020 ± 0.000	2.000 ± 0.000	8.216 ± 0.470
AE-8	0.008 ± 0.006	0.500 ± 0.000	25.800 ± 1.493	8.757 ± 0.491	40.067 ± 1.570	1.573 ± 0.993	0.549 ± 0.151	146.667 ± 10.610	0.604 ± 1.011	0.603 ± 1.011	19.167 ± 6.331	8.010 ± 0.173
AE-9	0,059 ± 0,067	0.967 ± 1.053	23.928 ± 3.186	9.488 ± 3.122	38.087 ± 4.532	2.762 ± 2.803	0.564 ± 0.931	147.231 ± 11.416	0.299 ± 0.577	0.299 ± 0.577	12.461 ± 5.838	8.009 ± 0.429
AE-10	0.015 ± 0.011	1.392 ± 1.322	24.300 ± 3.471	8.698 ± 0.911	37.945 ± 2.568	3.673 ± 5.445	0.514 ± 0.404	147.381 ± 7.550	0.315 ± 0.616	0.315 ± 0.616	2.054 ± 1.935	8.028 ± 0.415
AE-11	0.057 ± 0.071	0.995 ± 1.160	24.439 ± 4.139	8.921 ± 0.587	37.890 ± 2.916	1.727 ± 1.394	0.448 ± 0.360	146.987 ± 7.300	0.289 ± 0.600	0.289 ± 0.600	10.682 ± 7.056	8.074 ± 0.394
KD-3	0.005 ± 0.000	1.544 ± 1.587	22.956 ± 3.717	8.852 ± 0.830	16.794 ± 1.044	10.978 ± 0.780	0.206 ± 0.134	170.489 ± 7.402	11.162 ± 20.657	0.023 ± 0.007	6.489 ± 4.328	8.438 ± 0.168
KD-4	0.018 ± 0.038	1.330 ± 1.823	23.500 ± 3.604	8.994 ± 1.132	16.517 ± 1.671	8.325 ± 0.564	0.169 ± 0.099	170.378 ± 68.890	16.129 ± 23.552	0.031 ± 0.019	6.144 ± 2.310	8.466 ± 0.257
KD-5	0.005 ± 0.000	2.019 ± 1.878	23.017 ± 4.219	10.148 ± 4.798	16.628 ± 1.178	12.000 ± 2.123	0.197 ± 0.130	171.317 ± 11.930	4.652 ± 14.917	0.029 ± 0.029	5.071 ± 2.918	8.548 ± 0.218
KD-7	0.007 ± 0.010	0.982 ± 1.201	23.561 ± 3.180	8.772 ± 0.909	16.897 ± 0.983	11.787 ± 1.466	0.189 ± 0.142	178.356 ± 28.266	10.845 ± 15.705	0.033 ± 0.019	5.656 ± 2.724	8.357 ± 0.263
MD-1	0.007 ± 0.004	1.936 ± 2.231	20.622 ± 4.536	10.754 ± 4.301	21.555 ± 5.976	1.000 ± 0.000	0.327 ± 0.268	185.600 ± 27.093	1.945 ± 5.034	0.063 ± 0.096	3.633 ± 1.990	8.300 ± 0.272
MD-2	0.006 ± 0.002	2.020 ± 1.877	20.222 ± 3.781	9.003 ± 0.883	25.611 ± 6.422	1.000 ± 0.000	0.303 ± 0.295	167.956 ± 12.246	2.374 ± 4.285	0.087 ± 0.079	3.533 ± 1.931	8.338 ± 0.167
MD-4	0.006 ± 0.002	2.526 ± 1.971	20.655 ± 4.316	9.011 ± 0.810	23.372 ± 6.279	1.000 ± 1.155	0.389 ± 0.477	167.000 ± 18.459	2.562 ± 4.771	0.227 ± 0.494	3.591 ± 1.938	8.341 ± 0.125
MD-6	0.007 ± 0.005	2.245 ± 1.959	20.691 ± 3.717	8.965 ± 0.965	24.603 ± 4.930	4.654 ± 7.191	0.249 ± 0.278	170.627 ± 18.766	2.415 ± 4.129	0.059 ± 0.070	5.257 ± 2.965	8.304 ± 0.246

*TP: Total phosphorus (mg/L), CHL: Chlorophyll-a (µg/L), T: ℃, DO: Dissolved Oxygen (mg/L), S: Salinity (٪), TOC: Total Organic Carbon (mg/L), TN: Total Nitrogen (mg/L), ALK: Alkalinity, SI: Silica (mg/L), DIN: Dissolved Inorganic Nitrogen (mg/L), SD: Sechhi Depth (m)

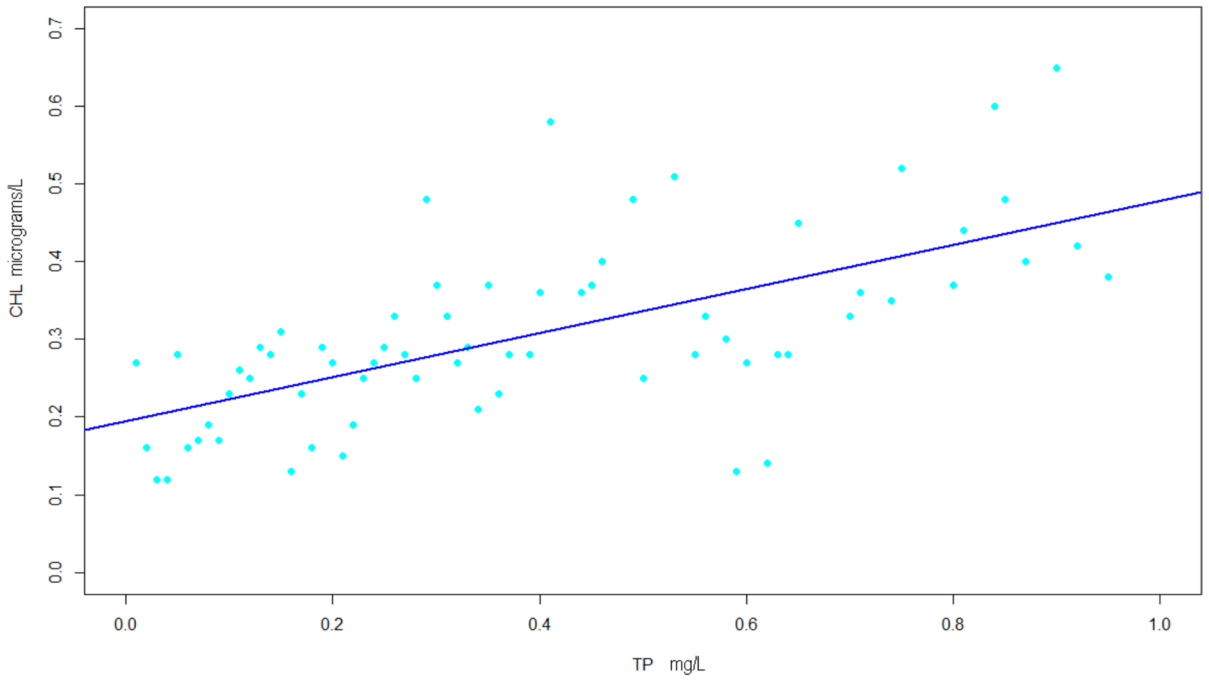

Fig. 2 Linear regression between Chl-a (µg/L) and TP (mg/L) values measured from the samples. The model is represented as: CHL = 0.195 + 0.28TP + 0.05. Model fit numbers are R-squared: 0.4056, F-statistic is 44.36 on 1 and 65 degrees of freedom, with a p-value of 6.873e-09

the Baltic Sea and elsewhere, it has been well documented that intensive nutrient loadings result in a rapid decline in SD levels when phytoplankton concentrations reach a peak (Chen et al., 2023). The average SD values, in our study, are broadly in line with previous study findings in the Black Sea and Mediterranean regions. For the Black Sea, historical SD values mostly range between 5 and 20 m, depending on the location (Man'kovsky & Zemlyanaya, 1989). In general, SDs in the open sea are much higher than those in coastal zones. Factors, such as blooms of phytoplankton, suspended sediments, and river inputs, particularly from the Danube and other rivers, significantly reduce the SD values nearshore (Vedernikov, 1991). Moreover, Rumuri et al. (2022) emphasize that sediment grain size parameters have a particular influence on phytoplankton richness in estuarine environments. This phenomenon is reflected by our low values of the types KD3 and KD7, which are quite in concord with the SD values registered in studies around the highly productive and turbid nearshore zones of the Black Sea. The overall pattern of SD that we have described conveys the variable environmental conditions within the Black Sea and Mediterranean.

Chlorophyll is essential for capturing light energy for photosynthesis, which drives phytoplankton growth and energy production (Falkowski & Raven, 2007). As a primary pigment, Chl-a is vital for photosynthesis and serves as an indicator of phytoplankton biomass and productivity in marine ecosystems. Low Chl-a concentrations in AE-1 and AE-8 (Mediterranean Sea) suggest low phytoplankton biomass, which is typical of oligotrophic seas where limited nutrient availability constrains primary production (Lloret et al., 2017). Conversely, elevated Chl-a levels in AE-7 (Mediterranean Sea) point to a localized increase in productivity, potentially driven by nutrient influx or favorable oceanographic conditions such as upwelling or riverine sources (Mayot et al., 2017). Similarly, Chl-a levels in KD-5 and KD-7 (Black Sea) align with the productivity patterns of the Baltic and Black Seas (Carstensen et al., 2014a, 2014b).

The relationship between Chl-a and TP serves as a key indicator of phytoplankton biomass and eutrophication, holding significant implications for water

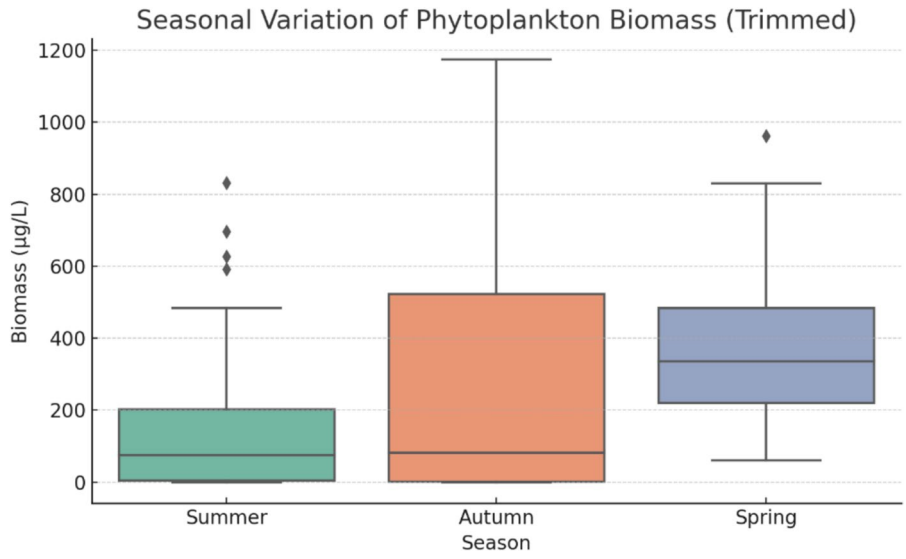

Fig. 3 Seasonality in phytoplankton biomass. In order to minimize the influence of outliers, a trimmed dataset (5th–95th percentile) was used. The maximum value was observed in the spring season, 14 mg/L

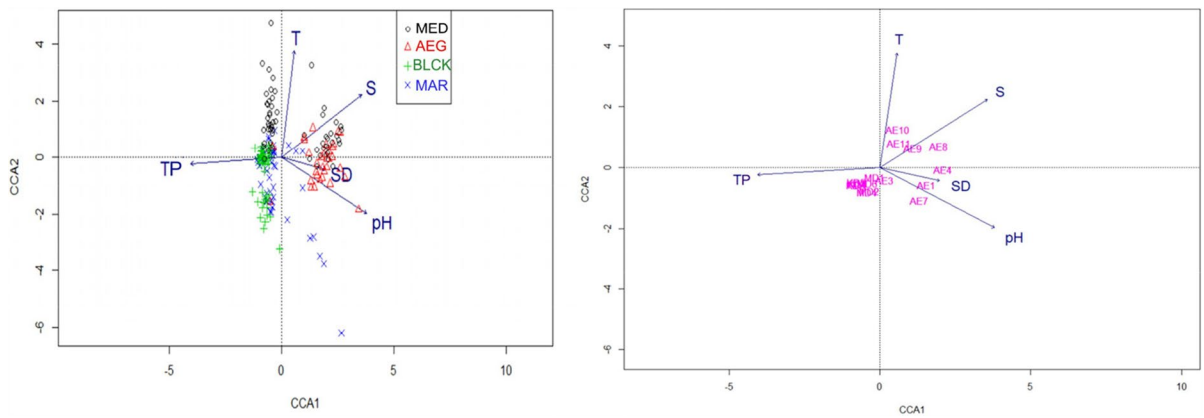

Fig. 4 CCA analysis of phytoplankton biomass data constrained by environmental factors

quality management. Typically, Chl-a levels correlate positively with TP concentrations, a relationship that is utilized to assess the eutrophication status of aquatic systems. Elevated TP levels often result in increased phytoplankton biomass, which is reflected in higher Chl-a concentrations (Filstrup & Downing, 2017). The observed positive association between TP and Chl-a highlights the fundamental role of nutrient enrichment in regulating phytoplankton production. While TP is identified as a statistically significant driver of Chl-a, its moderate explanatory capacity ($R^2 \approx 0.40$) indicates that a substantial portion of the variability in biomass remains unaccounted for. This suggests that phosphorus enrichment alone does not fully explain phytoplankton biomass dynamics in Turkish coastal waters. Additional contribution factors likely include physical processes such as vertical mixing, hydrodynamic transport, light penetration, and water column stratification; chemical influences such as nitrogen-to-phosphorus ratios, silica concentrations essential for diatom growth, and trace metals (e.g., iron) affecting nutrient uptake; and biological processes including zooplankton grazing, shifts in species composition, and episodic harmful algal bloom events. Climatic variability-particularly seasonal wind regimes and storm-induced mixing-may further modulate biomass independently of TP concentrations. From a management standpoint, these findings emphasize the importance of the multi-metric monitoring strategies that incorporate physical, chemical,

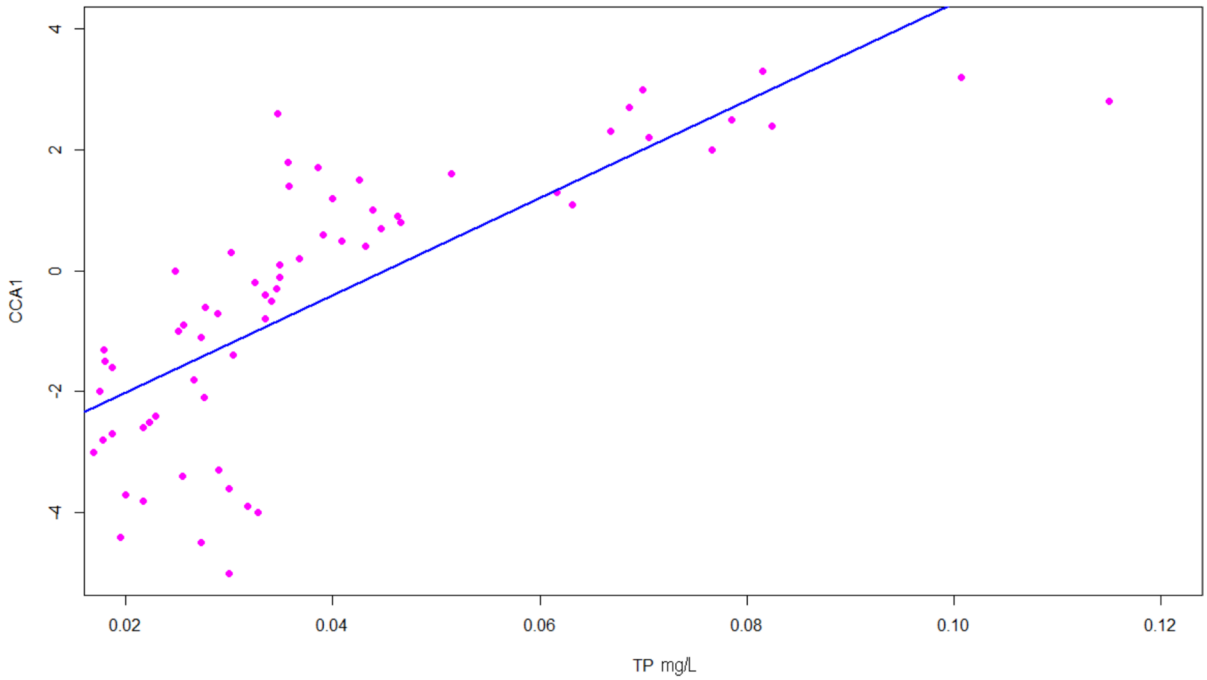

Fig. 5 Linear regression between CCA1 and TP (mg/L) values measured from the samples. The model is represented as: CCA1 = −3.631 + 80.566xTP + 8.544. Model fit numbers are R-squared: 0.5853, F-statistic is 88.91 on 1 and 63 degrees of freedom, with a p-value of 1.179e-13

and biological indicators alongside TP. Broadening the scope of monitored parameters has the potential to enhance the predictive capacity of models and improve the accuracy of ecological status evaluations within the framework of the WFD. Similar findings, with an explained variance of 38% were observed in the Baltic Sea by Osowiecki et al. (2012). In the Western Black Sea, the relationship between TP and Chl-a weaker, explaining only 13.67% of the variance (Moncheva & Boicenco, 2011). However, a stronger regression (76.2%) was reported for Type IIA water bodies in the Mediterranean (Gionavardi et al., 2018), which may be attributed to the analysis being limited to a single type of water.

The identified spatial and temporal patterns carry important implications for management within the framework of the WFD. In the Black Sea and Marmara types, pronounced spring biomass peaks indicate that nutrient reduction interventions should be implemented prior to the spring growth phase to maximise their effectiveness. These peaks coincide with periods of elevated risk for harmful algal blooms (HABs), marking windows for intensified monitoring.

Table 3 Reference conditions and class boundaries of the chl-a data (µg/L)

Types	REF	H/G	G/M	M/P	P/B
AE-1,3,4,7	0.01	0.02	0.04	0.06	0.08
AE-8,9	0.01	0.02	0.05	0.07	0.10
AE-10	0.11	0.22	0.44	0.67	0.89
AE-11	0.09	0.18	0.37	0.55	0.74
KD-3,4	0.67	1.35	2.69	4.04	5.38
KD-5	0.24	0.48	0.95	1.43	1.91
KD-7	0.10	0.20	0.39	0.59	0.79
MD-1,2	0.33	0.66	1.33	1.99	2.66
MD-4	0.58	1.17	2.33	3.50	4.66
MD-6	0.35	0.70	1.39	2.09	2.79

In contrast, for the Aegean and Mediterranean types-where baseline biomass remains low-monitoring should prioritise the detecting of short-lived productivity events, such as the fall peak observed in AE-7, which may reflect localised eutrophication or a typical hydrodynamic conditions. Accounting for typology-specific responses enables management strategies to

be more precisely targeted and cost-efficient, thereby avoiding both underestimation and overestimation of ecological risks.

Uncertainties and limitations in phytoplankton monitoring protocols play a central role in shaping the reliability of reference conditions, which in turn are critical for policy evaluations under the Water Framework Directive (WFD). Seasonal sampling, as commonly used in WFD monitoring, can significantly overestimate phytoplankton biomass and fail to capture short-term community shifts caused by extreme weather events, highlighting the need for higher-frequency and methodologically diverse sampling strategies (Bergkemper & Weisse, 2018). Solheim et al., 2008 highlights that inconsistencies in monitoring methods, lack of data standardization, and mismatches between monitoring systems have significant impacts on measurement performance and the comparability of ecological status classifications. Uncertainties in phytoplankton indicators—rooted in sampling variability, analytical biases, and natural temporal and spatial variation—can mask true ecological trends under shifting climatic and anthropogenic pressure regimes, thereby risking inaccurate ecological status assessments and misinformed policy responses (Salmaso et al., 2018; Thackeray et al., 2013) Therefore, enhancing the precision and standardization of phytoplankton monitoring is not only a technical necessity but also a policy imperative.

Establishing reference conditions under the WFD is a complex process that requires region-specific approaches. The integration of spatial data, modelling, historical records, and expert judgment, supported by intercalibration efforts, ensures consistent and reliable ecological assessments across Europe (Heiskanen et al., 2004). Continuous refinement of these methods and cooperation among member states are crucial for achieving the WFD's goals of protecting and enhancing water quality. For instance, in Northern Europe, reference conditions are determined through the use of historical data and ongoing monitoring. Key initiatives such as Denmark's National Aquatic Monitoring/Assessment Program and the Helsinki Commission (HELCOM) provides essential guidance in establishing reference conditions in the Baltic Sea (Conley et al., 2002; Andersen et al., 2006). The Chl-a 90th percentile is an important metric for identifying eutrophication and deviations from natural conditions (MED-GIG Report, 2011).

Percentile-based class boundaries serve not only as statistical benchmarks but also as operational management thresholds. Surpassing the high/good (H/G) boundary can prompt the implementation of preventative nutrient control measures, whereas exceeding the good/moderate (G/M) threshold may necessitate active remediation in accordance with the WFD. Elevated boundary values recorded in naturally productive Black Sea types (e.g., KD-7) underscore the importance of establishing realistic, type-specific targets to prevent the misclassification of inherently eutrophic systems as ecologically degraded. In contrast, the comperatively low boundaries characteristic of oligotrophic Aegean types (e.g., AE-1) ensure that even minor nutrient-induced biomass increases are detected at an early stage. Comperable type-specific calibration strategies have been adopted in MED-GIG and Baltic-GIG intercalibration exercises, where percentile-based thresholds were adjusted to reflect natural productivity gradients, thereby enhancing both ecological validity and management feasibility.

In Western Europe, reference conditions are based on a combination of historical phytoplankton data and current monitoring efforts, utilizing metrics like Chl-a levels, nutrient concentrations, and species diversity to set reference benchmarks (Gamito, 2008; Devlin et al., 2007). The Mediterranean region adapted WFD methodologies to suit its unique environmental conditions, relying on localized historical data and current observations to establish reference conditions. Metrics such as Chl-a concentrations, nutrient levels, and phytoplankton diversity are tailored to the specific characteristics of the Mediterranean coastal waters.

Table 4 Reference conditions and class boundaries of the phytoplankton biomass data (µg/L)

Types	REF	H/G	G/M	M/P	P/B
AE-1,3,4,7	9	17	26	35	43
AE-8,9	35	70	105	140	176
AE-10	43	86	129	173	216
AE-11	31	62	92	123	154
KD-3,4	74	149	223	298	372
KD-5	110	219	329	438	548
KD-7	274	547	821	1095	1369
MD-1,2	14	28	42	56	70
MD-4	103	207	310	413	516
MD-6	90	180	270	359	449

In the Black Sea, reference conditions for Romanian coastal waters have been informed by palaeoecological data, which provide a baseline for understanding past ecological conditions. In Türkiye, water body types were identified through the DEKOS Project (DGEM, 2014). Following this, the process of establishing reference conditions began with selecting of relatively unimpacted sites representing different water body types in this study (Establishment of Reference Monitoring Network in Türkiye Project). After a 6-year biomonitoring program, reference sites were assessed using EQR and expert judgements.

A series of Geographic Intercalibration Groups (GIGs), such as MED-GIG, Baltic Sea-GIG, and Black Sea Coastal GIG, were conducted alongside the REFCOND procedures (Carletti & Heiskanen, 2009). These GIGs aimed to implement and harmonize coastal phytoplankton metrics across various countries. Despite testing multiple tools, Chl-a was the only metric universally agreed upon by all GIGs for phytoplankton assessment. However, significant data gaps caused by inconsistent historical data and varying monitoring practices across countries present challenges in establishing accurate reference conditions. While Chl-a is a useful proxy for nutrient pressures, it does not fulfil all the requirements of the WFD. Chl-a alone cannot account for species composition, abundance, or episodic events, which are critical for assessing ecosystem health (GIG Black Sea 2011). Changes in species composition and abundance, which indicate shifts due to nutrient enrichment or other environmental stressors, cannot be detected by only Chl-a. Additionally, Chl-a measurements provide only snapshots and may miss episodic events like harmful algal blooms (HABs), which have significant ecological and economic impacts.

The MED-GIG also acknowledged limitations of using Chl-a as a sole metric. The report noted, "While Chl-a is a good proxy for nutrient pressures, it cannot fully capture the complexity of phytoplankton community dynamics and other biological quality elements (BQEs) required by the WFD" (MED-GIG Report, 2011). In this study, we found that both Chl-a (R-squared = 0.42) and phytoplankton biomass metrics are sensitive to nutrients. However, the latter showed a stronger relationship with eutrophication levels. By using CCA constrained on biomass, we achieved higher R^2 values (R-squared = 0.59). The CCA results indicate that TP-driven eutrophication and temperature–salinity regimes are the principal factors shaping phytoplankton biomass patterns in Turkish coastal waters. The typological separation along CCA1 was statistically significant (ANOVA, $p = 0.036$) and aligned with established nutrient gradients distinguishing the Black Sea/Marmara from the Aegean/Mediterranean regions. Although the clustering of types was largely well-defined, partial overlap between certain Aegean and Mediterranean subtypes suggests the presence of transitional conditions or common environmental drivers, a pattern also documented in studies of the Baltic and Adriatic Seas (Olenina et al., 2006; Giovanardi et al., 2018; Rivaro et al., 2004). Moreover, Pan et al. (2025) presented a technological approach to phytoplankton monitoring, which complements the use of Canonical Correspondence Analysis (CCA) within the scope of this study.

Our findings are generally consistent with patterns reported in other national and regional reference condition assessments. For instance, the relatively high biomass thresholds identified for Turkish Black Sea types correspond closely with those reported for the northern Black Sea GIG (Moncheva and Boicenco, 2011), whereas the low thresholds in Aegean types mirror those observed in oligotrophic typologies of the MED-GIG (Ciancia et al., 2021). Comparable percentile-based boundary-setting methods have also been employed in the Baltic-GIG (Fleming-Lehtinen et al., 2015), though with greater emphasis on phenological indicators due to pronounced seasonal variability. These parallels indicate that our typology-specific approach aligns well with established EU methodologies, thereby supporting meaningful international benchmarking and facilitating integration into broader intercalibration initiatives.

5 Conclusion

Seasonal variations revealed distinct patterns in nutrient and phytoplankton levels across various stations, underscoring the importance of localized monitoring and assessment in marine environments. Beyond Chl-a, measuring phytoplankton biomass is also crucial for evaluating the ecological status of coastal waters. CCA on biomass data showed that the samples were distributed along the first axis, constrained primarily by eutrophication parameters,

particularly TP. Biomass, therefore, provides complementary insights that enhance our understanding of ecosystem health and productivity. Although Chl-a and total biomass are core indicators under the WFD, both have inherent limitations. Chl-a measurements do not provide taxonomic resolution and may overestimate biomass during the presence of non-algal turbidity, while total biomass estimates can be artificially elevated by detrital material or colonial forms, particularly following bloom collapse. Incorporating complementary techniques-such as high performance liquid chromatography (HPLC) for pigment profiling, automated cell enumeration, and molecular-based approaches-would enhance the accuracy and ecological relevance of reference condition assessments. This presents an opportunity to develop more explanatory indicators or indices. These could include integrated indices, functional indices, weighted average indices, or a combination of several such approaches. In additon to establishing reference conditions and class boundaries for the first time in Turkish coastal marine waters, this study provides valuable insights for the development and refinement of monitoring and management practices under the WFD. The typology-specific boundaries and seasonal biomass patterns identified can inform the design of targeted monitoring programmes, ensuring that sampling frequencies are optimised to capture key seasonal peaks. From a regulatory perspective, the High/Good and Good/Moderate boundaries can serve as enforcement triggers, initiating nutrient reduction measures when exceeded. Embedding these boundaries within adaptive management frameworks would allow for dynamic adjustment of monitoring intensity and mitigation strategies in response to deviations from reference conditions, thereby enhancing both operational efficiency and ecological effectiveness.

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Author Contributions Özgür Baytut: Conceptualization, Methodology, Data Analysis, Writing – Original Draft.

Tolga Çetin: Project Coordination, Resources, Validation, Writing – Review and Editing.

Fatma Çolak Sabancı: Phytoplankton Identification, Visualization, Review and Editing.

Fatih Gümüş: Sampling and Laboratory Analysis, Statistical Analysis, Review and Editing.

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Data Availability The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request. Some data may also be accessed through the Ministry of Agriculture and Forestry upon official application, due to project-based data governance policies.

Declarations

Ethics Approval and Consent to Participate This study was conducted using environmental monitoring data obtained under the "Establishment of Reference Monitoring Network in Türkiye" project (2016–2020), coordinated by the Ministry of Agriculture and Forestry, Directorate General of Water Management. As the study did not involve human participants, animals, or any private data, no specific ethical approval was required.

Consent for Publication All authors have reviewed and approved the final version of the manuscript and consent to its submission and publication in Water, Air, and Soil Pollution.

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

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