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A new diatom index to assess ecological quality of running waters: a case study of water bodies in western Anatolia

Assane Anabi Toudjani[1], Abuzer Çelekli[1]*, E. Yonca Gümüş[1], Seda Kayhan[1], H. Ömer Lekesiz[1] and Tolga Çetin[2]

[1] Department of Biology, Faculty of Art and Science, University of Gaziantep, 27310 Gaziantep, Turkey
[2] T.R. Ministry of Forestry and Water Affairs, Directorate General for Water Management, Ankara, Turkey

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Abstract – Diatoms are an important part of aquatic biodiversity and the main component of phytobenthos. They play a key role in aquatic ecosystems and indicate water quality. The European Union Water Framework Directive requires that phytobenthos be used for the ecological quality assessment of water. This study evaluated the ecological status of various watercourses in the western Mediterranean basin of Turkey using a multivariate approach and presents a new trophic index Turkey (TIT) based on diatom assemblages. Twenty-five running water bodies were seasonally monitored for biological and physicochemical analyses from summer 2014 to summer 2015. A total of 102 species belonging to 22 genera were recorded. *Cymbella excisa* Kützing, *Gomphonella parvula* (Kützing) Rabenhorst, *Ulnaria ulna* (Nitzsch) Compère, and *Cocconeis communis* f. *placentula* (Ehrenberg) Chmielevski were the most commonly found species. A canonical correspondence analysis was used to examine the relationship between species and environmental factors. The most effective explanatory factors, including nitrate nitrogen, electrical conductivity, altitude, total nitrogen, orthophosphate, and calcium carbonate significantly influenced the ecological preferences of diatom species in the ecosystems. TIT values ranged between 1.53 in Kaya creek and 2.73 in Dalaman stream (A8). Ecological status of water bodies was assessed using an ecological quality ratio based on trophic index Turkey (EQR-TIT) for each of the stations. EQR-TIT ranged from 0.44 in Dalaman stream (A8) to 0.99 in Kocabük creek during the study period. TIT had a high correlation coefficient ($R^2 = 0.77$) with log total phosphorus and may be an appropriate diatom metric to assess the ecological status of water bodies.

Key words: Phytobenthos / EQR / TIT / Water Framework Directive / Water quality

Introduction

Water quality is an important parameter in drinking water supply, irrigation, fish production, recreation and other purposes for which the water was impounded (Mustapha, 2008; Wallis *et al.*, 2011). Since the industrial revolution, freshwater quality has deteriorated dramatically due to population growth, industrialization and climate change, leading to severe water pollution and scarcity worldwide (Schindler, 2006; Smol, 2008). Direct sewage inputs, runoff from fertilized soils and land erosion through logging activities have had a major impact on water resources over the last century (Billen *et al.*, 2001; Wunsan *et al.*, 2002; Ducharne *et al.*, 2007; Delgado and Pardo, 2014). The increasing availability of nutrients such as nitrate and phosphate (through fertilizers or sewage) in freshwaters, commonly associated with eutrophication, is affecting the productivity and community structure of primary producers in aquatic ecosystems (Leira *et al.*, 2009; Delgado and Pardo, 2014).

In recent decades, significant efforts have been made in many countries to assess water quality using not only physical and chemical parameters but also biological elements (Rott *et al.*, 1999; Padisák *et al.*, 2006; Kelly *et al.*, 2008; Mustapha, 2008). In accordance with the European Union Water Framework Directive (WFD), biological quality elements, including phytoplankton, phytobenthos (especially benthic diatoms), macrophytes, benthic invertebrates, and fish, should be employed as ecological indicators for the assessment of surface waters (Directive, 2000; European Communities, 2009). Among these biological groups, diatoms are widely used in bioassessments due to their short life cycles and rapid response to different stressors (Hering *et al.*, 2006; Bona *et al.*, 2007; Rimet, 2012; Delgado and Pardo, 2014). In this respect, a large number of diatom indices

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*Corresponding author: `celekli.a@gmail.com`

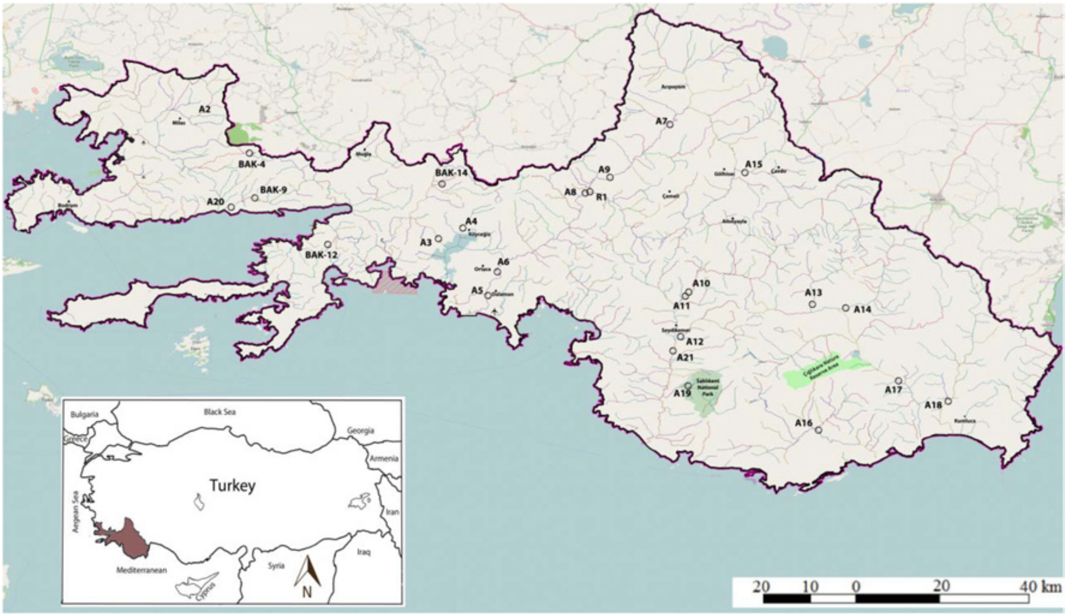

Fig. 1. Location of sampling stations. For station codes: Sarıçay creek, A2; Namnam creek, A3; Dalaman stream, A5–9; Seki stream, A10, A11; Çayiçi creek, A12; Kızılöz creek, A13; Kocadere creek, A14; Çavdır stream, A15; Boğluca stream, A16; Akçay stream, A17; Alakır stream, A18; Eşen stream-1, A19; Kanlı stream, A20; Eşen stream-2, A21; Kaya creek, BAK4; Kocabük creek, BAK9; Karabeyyurdu creek, BAK12; Delin creek, BAK14; R1 creek, R1.

have been developed to estimate water quality in various geographic areas (Potapova *et al*., 2004).

The studies that used phytobenthos (hereafter related specifically to diatoms) to monitor aquatic ecosystems have mainly investigated running waters (*e.g*. Round, 1991; Vadeboncoeur and Steinman, 2002; Potapova and Charles, 2003; Rott *et al*., 2003; Ács *et al*., 2004; Bellinger *et al*., 2006; Della Bella *et al*., 2012; O'Driscoll *et al*., 2012; Rimet, 2012; Delgado and Pardo, 2014; Leelahakriengkrai and Peerapornpisal, 2014). In this regard, several diatom indices have been developed in different countries to evaluate the ecological status of streams and rivers, including the saprobic index Zelinka and Marvan (1961), *indice de polluosensibilité* ≈ the specific pollution index, SPI (CEMAGREF, 1982), the trophic index, TI (Rott *et al*., 1999), the Eutrophication and/or Pollution Index – Diatom based, EPI-D (Dell'Uomo, 1999, 2004) and the trophic diatom index (TDI) (Kelly and Whitton, 1995; Kelly *et al*., 2008).

No diatom index for freshwater quality assessment is yet available for Turkish rivers. The direct adoption of foreign diatom index scores can lead to erroneous interpretation of water quality because there are limited overlaps in species composition across different regions (Pan and Stevenson, 1996). The aims of the study were to: (i) define the most important structuring factors of diatom species composition, and (ii) test the suitability and applicability of a recently developed diatom-based metric called the TIT as a potential tool for the ecological status assessment of Mediterranean running water bodies and its performance compared with EPI-D (Dell'Uomo, 1999, 2004), an index developed in another Mediterranean country, Italy.

Materials and methods

Study area and sampling

The studied water bodies were located in the western Mediterranean basin of Turkey (Fig. 1). Water and diatom samples were collected seasonally from 25 running water bodies from summer 2014 to summer 2015. Environmental variables such as water temperature, pH, electrical conductivity (EC), and salinity were measured *in situ* using a YSI professional plus oxygen–temperature meter from just below the surface water. Geographical data (altitude, latitude, and longitude) were recorded using a geographical positioning system (Garmin Vista HCx model GPS) during the study period. The water flow was measured using a StreamPro ADCP (Acoustic Doppler Current Profiler). The epilithic diatoms were collected according to European standard sampling methods for limnetic systems (Kelly *et al*., 1998; European Committee for Standardization, 2003, 2004a, b; European Communities, 2009). Epilithic samples were collected in riffle areas with normal hydrological condition (not disturbed) by scraping the upper surface of the stones using a toothbrush. The samples were fixed using Lugol's iodine solution immediately after collection (European Committee for Standardization, 2004a, b).

Laboratory techniques

Water samples were stored in iceboxes with ice packs and transferred to the laboratory for later analyses. Chemical variables such as total nitrogen (TN), ammonium nitrogen ($N\text{-}NH_4$), nitrate nitrogen ($N\text{-}NO_3$), nitrite nitrogen ($N\text{-}NO_2$), total phosphorus (TP), orthophosphate ($P\text{-}PO_4$), biological oxygen demand (BOD_5), and calcium carbonate ($CaCO_3$) were measured using the standard methods (APHA, 2012).

Permanent slide mounts were prepared for each sample using the standard methods (Kelly *et al.*, 1998; European Committee for Standardization, 2004a, b; European Communities, 2009) to enumerate diatom species. The algae were later examined at 1000 × magnification to estimate the relative abundance of taxa using a compound microscope (Olympus BX53) equipped with a digital camera (DP73 model) and imaging software (Olympus CellSens Vers. 1.6). Diatoms were identified to species level using taxonomic keys (Krammer and Lange-Bertalot, 1991a, b, 1999a, b; Krammer, 2000, 2002; Lange-Bertalot, 2001). Relative percentages of diatom species were calculated and used for statistical analyses and assessment of water quality. To develop the TIT index (Çelekli *et al.*, 2017), data collected from 225 running water bodies from eight basins of Turkey between 2014 (summer and fall) and 2015 (spring and summer) were used. A total of 226 diatom species were identified and the percentages of each species at each sampling station were to be determined. According to the distribution of diatom species based on TP classes, trophic weight and indicator values were determined as described by Rott *et al.* (1999) and Binder (2001). The TIT value was calculated using the following equation:

$$TIT = \frac{\sum_{i=1}^{n} b_i \times e_i \times c_i}{\sum_{i=1}^{n} e_i \times c_i} \tag{1}$$

where, b_i: i-taxon trophic weight based on TP classes (1–5); e_i: i-taxon indicator value (0–5); and c_i: the percentage of valves of i-species in a sample.

TIT values ranging from 0 to 4 were then expressed as ecological quality ratios (EQRs) for the ecological quality assessment of running water bodies according to the WFD. Boundary classes were deteremined according to the WFD, using the box-plot analysis for each typology (Çelekli *et al.*, 2017). EQR is given by (Equation (2))

$$\text{EQR} = \frac{(4 - \text{TIT}_o)}{(4 - \text{TIT}_{ref})} \tag{2}$$

where TIT_o and TIT_{ref} are the observed and reference TIT, respectively. The EQR values ranged from 0 (poor ecological status) to 1 (high ecological status).

TIT was then compared with the Eutrophication and/or Pollution Index – Diatom based (EPI-D) (Dell'Uomo, 1999, 2004) to evaluate its ability to assess water quality.

Multivariate analyses

Detrended correspondence analysis (DCA), which is a unimodal response before performing CCA (Leps and Smilauer, 2003) was used at a gradient length more than 3.0. A direct gradient analysis technique, canonical correspondence analysis (CCA), was used to examine the relationship between the response variables (diatoms) and predictor variables (environmental factors) in 25 running water bodies (ter Braak and Šmilauer, 2002). Environmental variables were transformed ($\ln(x+1)$, except for pH) to decrease skewness (ter Braak and Šmilauer, 2002). A total of eight environmental variables were used for CCA. The significance of environmental variables to clarify the variance of diatom data in CCA was tested using the forward selection of Monte Carlo simulations with 499 unrestricted permutations. Only species weight range with a percentage of over 1% were selected for the CCA diagram. CCA was performed using the CANOCO program (ter Braak and Šmilauer, 2002).

Results

Physicochemical variables

The results for environmental variables at the sites are given in Table 1. The water bodies had alkaline water with pH ranging between 8.1 and 8.7. Delin creek had the lowest mean temperature (14.2 °C), whereas Çayiçi creek had the highest (23.8 °C).

With regard to nutrients, Dalaman stream (A8) had the highest mean values for TN (4029.7 $\mu g.L^{-1}$), $N\text{-}NO_3$ (1503.3 $\mu g.L^{-1}$), TP (783.3 $\mu g.L^{-1}$), and $P\text{-}PO_4$ (341.7 $\mu g.L^{-1}$), while the lowest values for TN (149.5 $\mu g.L^{-1}$), $N\text{-}NO_3$ (100 $\mu g.L^{-1}$), and $P\text{-}PO_4$ (10 $\mu g.L^{-1}$) were measured in Kaya creek (Bak4), followed Kocabük and Kanlı streams.

The Dalaman stream had a permanent and highest water flow (*e.g.* 36.8 $m^3.s^{-1}$ at A6 and 34.8 $m^3.s^{-1}$ at A5). The highest salinity (0.43 ppt) and EC (776.5 $\mu S.cm^{-1}$) values were also found in Dalaman stream (A8), while the lowest values of 0.10 ppt and 179.4 $\mu S.cm^{-1}$, respectively, were measured in Sarıçay creek.

Diatom assemblages

A total of 102 species belonging to 22 genera mostly dominated by *Navicula* and *Cymbella* were recorded (Table 2). Some species such as *Fragilaria capucina* Desmazières, *Cymbella excisa* Kützing, *Gomphonella parvula* (Kützing) Rabenhorst, *Ulnaria ulna* (Nitzsch) Compère, and *Cocconeis communis* f. *placentula* (Ehrenberg) Chmielevski were commonly found in the watercourses during the study period. On the other hand, *Navicula scutelloides* var. *disculus* (Schumann) Torka, *Diploneis modica* Hustedt, and *Surirella ovata* var. *pinnata* (W.Smith) Hustedt were rarely observed.

Species–environment relationships with multivariate analyses

The unimodal response of diatom taxa to environmental variables is found with gradient length, which

Table 1. Mean ± SD (standard deviation) values for environmental variables combined from sampling stations. For station codes, see Fig. 1.

	Altit (m)	Temp (°C)	EC (µS.cm^{-1})	pH	BOD (mg.L^{-1})	TN (µg.L^{-1})	N-NH$_4$ (µg.L^{-1})	N-NO$_2$ (µg.L^{-1})	N-NO$_3$ (µg.L^{-1})	TP (µg.L^{-1})	P-PO$_4$ (µg.L^{-1})	Salinity (ppt)	CaCO$_3$ (mg.L^{-1})
A2	433	18.1 ± 4.1	179.4 ± 83.6	8.3 ± 0.6	14.5 ± 4.1	1037.3 ± 0.3	123.3 ± 0.0	10.7 ± 0.0	226.7 ± 0.1	443.3 ± 0.6	240.0 ± 0.3	0.10 ± 0.0	10.0 ± 0.0
A3	15	20.1 ± 5.0	535.5 ± 193.5	8.7 ± 0.3	8.93 ± 6.9	724.7 ± 0.3	128.3 ± 0.0	8.25 ± 0.0	262.5 ± 0.2	337.8 ± 0.4	179.5 ± 0.2	0.27 ± 0.1	30.0 ± 0.0
A4	36	20.7 ± 1.8	318 ± 1.8	8.7 ± 0.3	5.30 ± 3.8	194.4 ± 1.1	100.0 ± 0.0	2.0 ± 0.0	100.0 ± 0.8	50.0 ± 1.0	10.0 ± 0.8	0.17 ± 0.3	10.0 ± 1.4
A5	18	18.4 ± 6.5	495.0 ± 187.3	8.4 ± 0.4	15.8 ± 9.8	990.0 ± 0.1	193.0 ± 0.1	31.3 ± 0.0	390.0 ± 0.1	336.0 ± 0.4	175.5 ± 0.2	0.26 ± 0.1	25.0 ± 7.1
A6	31	18.0 ± 7.0	477.3 ± 204.8	8.4 ± 0.5	12.3 ± 7.6	936.0 ± 0.4	226.8 ± 0.2	9.15 ± 0.0	292.5 ± 0.1	357.5 ± 0.4	183.3 ± 0.1	0.26 ± 0.1	20.0 ± 0.0
A7	603	19.7 ± 6.4	627.8 ± 192.1	8.7 ± 0.3	7.93 ± 7.8	1786.3 ± 0.6	117.5 ± 0.0	34.5 ± 0.0	1307.5 ± 0.4	386.5 ± 0.6	192.8 ± 0.3	0.34 ± 0.1	27.5 ± 4.0
A8	821	16.1 ± 5.1	773.3 ± 385.6	8.3 ± 0.2	8.33 ± 4.3	4029.7 ± 2.3	1220.0 ± 1.2	223.3 ± 0.1	1503.3 ± 0.7	783.3 ± 0.8	341.7 ± 0.4	0.43 ± 0.2	25.0 ± 0.0
A9	710	19.4 ± 5.4	776.5 ± 305.4	8.6 ± 0.2	5.65 ± 2.1	1722.3 ± 0.9	125.0 ± 0.1	27.8 ± 0.0	1030.0 ± 0.7	374.3 ± 0.5	214.0 ± 0.2	0.42 ± 0.1	35.0 ± 7.0
A10	264	14.5 ± 1.6	331.7 ± 89.8	8.1 ± 0.4	5.07 ± 1.8	574.2 ± 0.3	126.7 ± 0.0	6.60 ± 0.0	306.7 ± 0.2	184.3 ± 0.2	137.3 ± 0.1	0.18 ± 0.0	20.0 ± 0.0
A11	200	16.0 ± 1.8	330.0 ± 102.6	8.5 ± 0.1	11.7 ± 12.1	940.5 ± 0.1	438.0 ± 0.3	81.5 ± 0.0	680.0 ± 0.3	240.5 ± 0.2	163.8 ± 0.1	0.16 ± 0.1	17.5 ± 3.5
A13	1060	17.8 ± 1.7	301 ± 1.3	8.5 ± 1.0	5.10 ± 1.9	755.0 ± 1.5	100.0 ± 1.1	12.1 ± 15	370.0 ± 1.7	64.0 ± 1.9	10.5 ± 1.8	0.17 ± 1.1	15.0 ± 1.8
A14	1043	14.6 ± 6.3	670 ± 61.5	7.4 ± 1.2	5.20 ± 1.3	1089.0 ± 1.1	100.0 ± 1.0	4.9 ± 2.8	140.0 ± 0.8	90.0 ± 3.8	23.0 ± 2.8	0.40 ± 0.8	20.0 ± 1.9
A12	151	23.8 ± 8.0	352.0 ± 75.1	8.7 ± 0.4	4.00 ± 0.0	615.8 ± 0.3	115.0 ± 0.0	4.78 ± 0.0	182.5 ± 0.2	164.0 ± 0.2	111.0 ± 0.1	0.18 ± 0.1	15.0 ± 0.0
A15	936	17.8 ± 4.2	623.3 ± 278.3	8.7 ± 0.2	10.8 ± 8.0	2208.8 ± 0.8	195.0 ± 0.1	33.0 ± 0.0	1340.0 ± 0.3	175.3 ± 0.2	106.3 ± 0.1	0.34 ± 0.0	32.5 ± 11.0
A16	135	18.7 ± 5.8	484.3 ± 289.8	8.4 ± 0.3	7.67 ± 6.3	486.4 ± 0.3	130.0 ± 0.1	2.83 ± 0.0	260.0 ± 0.3	596.3 ± 0.2	110.0 ± 0.2	0.24 ± 0.0	27.5 ± 11.0
A17	341	17.0 ± 1.4	281.5 ± 84.4	8.5 ± 0.2	5.00 ± 2.0	930.9 ± 0.6	125.0 ± 0.1	20.3 ± 0.0	632.5 ± 0.4	122.0 ± 0.1	105.0 ± 0.1	0.15 ± 0.0	12.5 ± 3.5
A18	64	19.3 ± 6.4	344.5 ± 24.7	8.2 ± 0.3	4.00 ± 0.0	479.8 ± 0.2	100.0 ± 0.0	3.45 ± 0.0	165.0 ± 0.1	38.5 ± 0.0	10.0 ± 0.0	0.17 ± 0.0	17.5 ± 7.1
A19	98	15.6 ± 1.1	319.8 ± 68.8	8.2 ± 0.3	5.50 ± 3.0	763.2 ± 0.3	112.5 ± 0.0	4.75 ± 0.0	480.0 ± 0.2	295.8 ± 0.4	183.8 ± 0.2	0.17 ± 0.0	25.0 ± 0.0
A20	44	20.7 ± 0.0	380.0 ± 0.0	8.1 ± 0.0	7.95 ± 0.0	254.8 ± 0.0	100.0 ± 0.0	3.00 ± 0.0	100.0 ± 0.0	30.0 ± 0.0	10.0 ± 0.0	0.20 ± 0.0	15.0 ± 0.0
A21	85	22.8 ± 5.4	439.0 ± 116.1	8.7 ± 0.4	8.42 ± 5.1	1265.0 ± 0.0	123.3 ± 0.0	23.8 ± 0.0	917.5 ± 0.0	170.5 ± 0.1	117.5 ± 0.0	0.22 ± 0.0	25.0 ± 0.0
Bak4	516	16.8 ± 0.0	234.0 ± 0.0	8.4 ± 0.0	9.50 ± 0.0	149.5 ± 0.0	100.0 ± 0.0	4.00 ± 0.0	100.0 ± 0.0	50.0 ± 0.0	10.0 ± 0.0	0.13 ± 0.0	15.0 ± 0.0
Bak9	86	16.4 ± 0.0	312.0 ± 0.0	8.3 ± 0.0	10.2 ± 0.0	208.9 ± 0.0	100.0 ± 0.0	3.00 ± 0.0	100.0 ± 0.0	40.0 ± 0.0	10.0 ± 0.0	0.18 ± 0.0	10.0 ± 0.0
Bak12	144	14.2 ± 0.0	563.0 ± 0.0	8.4 ± 0.0	4.00 ± 0.0	285.0 ± 0.0	100.0 ± 0.0	2.00 ± 0.0	100.0 ± 0.0	40.0 ± 0.0	10.0 ± 0.0	0.35 ± 0.0	30.0 ± 0.0
Bak14	375	18.2 ± 9.4	271.9 ± 87.8	8.6 ± 0.5	6.5 ± 12.3	609.9 ± 0.1	323.0 ± 0.3	21.0 ± 0.0	150.0 ± 0.1	54.5 ± 0.2	21.5 ± 0.0	0.15 ± 0.0	17.5 ± 3.6
R1	615	20.5 ± 5.5	504.0 ± 66.5	8.8 ± 0.2	4.41 ± 0.6	231.7 ± 0.1	100.0 ± 0.0	3.05 ± 0.0	130.0 ± 0.0	65.0 ± 0.0	10.0 ± 0.0	0.27 ± 0.0	27.5 ± 4.0

BOD, biochemical oxygen demand.

Table 2. List of diatom species and varieties found at the studied sites.

Species	Code	Species	Code
Achnanthes impexa (Lange-Bertalot)	*Acim*	*Dickieia expecta* (VanLandingham) D.G. Mann	*Diex*
Aulocoseira granulata (Ehrenberg) Simonsen	*Augr*	*Diploneis modica* (Hustedt)	*Dimod*
Bacillaria obtusa (W.Smith) Elmore	*Baob*	*Encyonema minutum* (Hilse) D.G. Mann	*Enmi*
Bacillaria vulgaris (Bory) Ehrenberg	*Bavu*	*Encyonema silesiacum* (Bleisch) D.G. Mann	*Ensi*
Clevamphora ovalis (Kützing) Mereschkowsky	*Clov*	*Epithemia adnata* (Kützing) Brébisson	*Epad*
Cocconeis communis f. *placentula* (Ehrenberg) Chmielevski	*Coco*	*Epithemia cistula* (Ehrenberg) Ralfs	*Epar*
Cocconeis placentula var. *lineata* (Ehrenberg) Van Heurck	*Coplv*	*Epithemia frickei* (Krammer)	*Epfr*
Cocconeis pumila (Kützing)	*Copu*	*Eunotia exigua* var. *tenella* (Grunow) Nörpel & Alles	*Euex*
Cocconeis scutellum (Ehrenberg)	Cosc	*Fragilaria biceps* (Ehrenberg)	*Frbi*
Cocconema helveticum (Kützing) West & G.S.West	*Cohe*	*Fragilaria capucina* (Desmazières)	*Frca*
Craticula accomoda (Hustedt) D.G. Mann	*Crac*	*Fragilaria dilatata* (Brébisson) Lange-Bertalot	*Frdi*
Craticula halophila (Grunow) D.G. Mann	*Crha*	*Gomphonema acuminatum* f. *laticeps* (Ehrenberg) Ant. Mayer	*Goac*
Cyclotella iris (Brun & Héribaud-Joseph)	*Cyir*	*Gomphonema angustum* (C. Agardh)	*Goan*
Cyclotella tibetana (Hustedt)	*Cyti*	*Gomphonema gracile* (Ehrenberg)	*Gogr*
Cymbopleura amphicephala (Nägeli) Krammer	*Cyam*	*Gomphonema minutum* (C. Agardh) C. Agardh	*Gomi*
Cymbopleura naviculiformis (Auerswald ex Heiberg) Krammer	*Cyna*	*Gomphonella parvula* (Kützing) Rabenhorst	*Gopa*
Cymatopleura solea (Brébisson) W. Smith	*Cyso*	*Gomphonema pseudoaugur* (Lange-Bertalot)	*Gops*
Cymbella cistula (Hemprich) Kirchner	*Cyci*	*Gomphonema truncatum* (Ehrenberg)	*Gotr*
Cymbella cymbiformis (Agardh)	*Cycy*	*Gomphonema truncatum* var. *clavatum* (Ehrenberg) Eberle	*Gotrc*
Cymbella ehrenbergii (Kützing)	*Cyeh*	*Halamphora subcapitata* (Kisselew) Levkov	*Hasu*
Cymbella excisa (Kützing)	*Cyex*	*Hantzschia distinctepunctata* var. *circuligera* (Compère)	*Hadi*
Cymbella hantzschiana (Krammer)	*Cyha*	*Hantzschia weyprechtii* (Grunow)	*Hawe*
Cymbella mesiana (Cholnoky)	*Cymes*	*Homoeocladia sigmoidea* (Nitzsch) Elmore	*Hosi*
Cymbella simonseni (Krammer)	*Cyst*	*Homoeocladia umbonata* (Ehrenberg) Kuntze	*Houm*
Cymbella tumida (Brébisson) van Heurck	*Cytu*	*Lysigonium lineatum* (Dillwyn) Trevisan	*Lyli*
Diatoma elongata subsp.*tenuis*(C. Agardh) Skabichevskii	*Diel*	*Lysigonium varians* (C. Agardh) De Toni	*Lyva*
Diatoma hyemalis (Roth) Heiberg	*Dihy*	*Navicula bryophila* (J.B. Petersen)	*Nabr*
Diatoma moniliformis (Kützing) D.M. Williams	*Dimo*	*Navicula capitatoradiata* (H. Germain)	*Naca*
Navicula cincta (Ehrenberg) Ralfs in Pritchard	*Naci*	*Placoneis clementis* (Grunow) E.J. Cox	*Plcl*
Navicula constans (Hustedt)	*Nacon*	*Pleurosigma acuminatum* (Kützing) Grunow	*Plac*
Navicula cryptocefalsa (Lange-Bertalo)	*Nacr*	*Scalptrum attenuatum* (Kützing) Kuntze	*Scat*
Navicula cryptocephala f. *Veneta* (Kützing) Hustedt	*Nacrv*	*Schizonema gregarium* (Donkin) Kuntze	*Scgr*
Navicula cryptonella (Lange-Bertalot)	*Nacry*	*Schizonema peregrinum* (Ehrenberg) Kuntze	*Scpe*
Navicula digitoradiata (Gregory) Ralfs in Prichard	*Nadi*	*Schizonema phoenicenterum* (Ehrenberg) Kuntze	*Scph*
Navicula heimansii (van Dam & Kooyman)	*Nahe*	*Schizonema radiosu* (Kützing) Kuntze	*Scra*
Navicula jaagii (Meister)	*Naja*	*Stephanocyclus meneghinianus* (Kützing) Skabitschevsky	*Stme*
Navicula lanceolata (C. Agardh) Kützing	*Nala*	*Surirella brebissonii* (Krammer & Lange-Bertalot)	*Subr*
Navicula margalithii (Lange-Bertalot)	*Nama*	*Surirella biseriata* f. *Amphioxys* (W. Smith) Hustedt	*Subi*
Navicula oppugnata (Hustedt)	*Naop*	*Surirella ovata* var. *Pinnata* (W. Smith) Hustedt	*Suop*
Navicula phylepta (Kützing)	*Naph*	*Surirella ovata* var. *ovalis* (Brébisson) Kirchne	*Suoo*
Navicula radians (Héribaud-Joseph)	*Nara*	*Surirella ovata* var. *angusta* (Kützing) A. Cleve	*Suon*
Navicula recens (Lange-Bertalot) Lange-Bertalot	*Nare*	*Surirella subsalsa* (W. Smith)	*Susu*
Navicula schoenfeldii (Hustedt 1930)	*Nasc*	*Ulnaria ulna* (Nitzsch) Compère	*Ulul*
Navicula scutelloides var.*disculus* (Schumann) Torka	*Nascu*		
Navicula slesvicensis (Grunow in van Heurck)	*Nasl*		
Navicula tripunctata (O.F. Müller) Bory	*Natr*		
Navicula trivialis (Lange-Bertalot)	*Natri*		
Navicula tuscula (Ehrenberg)	*Natus*		
Navicula vitabunda (Hustedt in Pascher)	*Navi*		
Naviculadicta laterostrata (Hustedt) Lange-Bertalot	*Nalat*		
Nematoplata crotonensis (Kitton) Kuntze	*Necr*		
Nitzschia angustata (W.Smith) Grunow	*Nian*		
Nitzschia brevissima (Grunow in Van Heurck)	*Nibr*		
Nitzschia elongata (Hassal)	*Niel*		
Nitzschia palea var. *dissipata* (Kützing) Schonfeldt	*Nipa*		
Nitzschia vermicularis var. *flexa* (Schumann) Cleve-Eule	*Nifl*		
Nitzschia vitrea var. *recta* (Hantzsch; Grunow) van Heurck	*Nivi*		
Odontidium elegans (Kützing) O'Meara 1875	*Odel*		

Table 3. Summary of canonical correspondence analysis (CCA) using a Monte Carlo permutation test for epilithic diatom species–environment variable relationships.

Axes	$\lambda1$	$\lambda2$	$\lambda3$	$\lambda4$	Total inertia
Eigen values	0.507	0.355	0.333	0.259	9.155
Species–environment correlations	0.906	0.894	0.865	0.823	
Cumulative percentage variance					
of species data	5.5	9.4	13.0	15.9	
of species–environment relations	24.6	41.8	58.0	70.5	
Sum of all eigen values					9.155
Sum of all canonical eigen values					2.060
Test of significance of first canonical axis: eigen value = 0.507					
		F-ratio = 3.222		*P*-value = 0.0240	

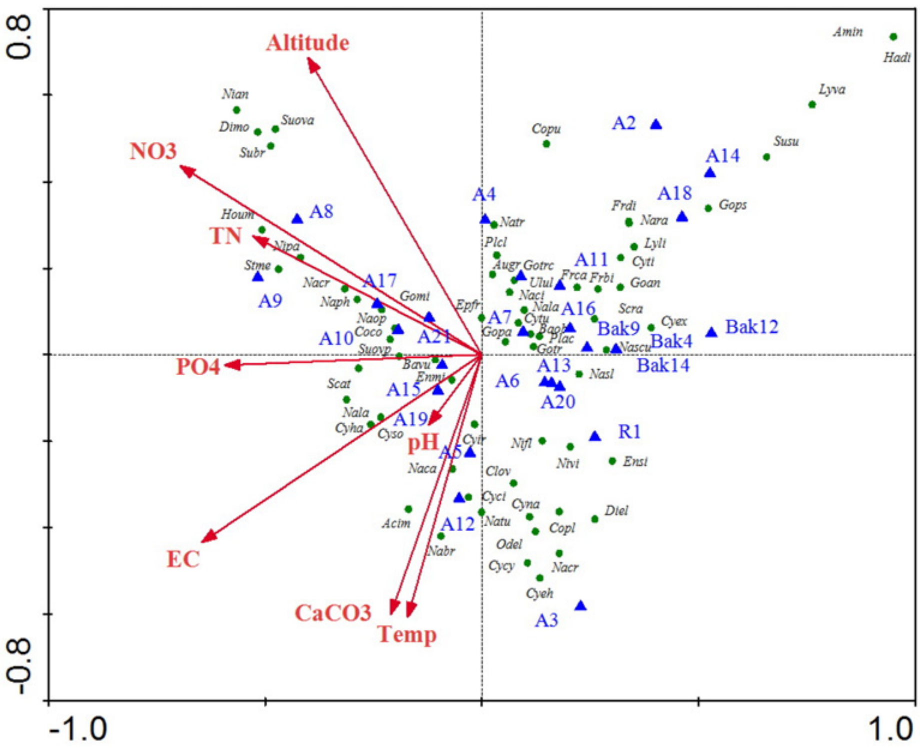

Fig. 2. CCA plot of species–environment relationships at the sampling stations (up triangular). For station codes, see Fig. 1 and for species, see Table 2.

was more than 3.0. After that, CCA analysis was performed to elucidate relationships between diatom species and environmental factors during the study period. 9.4% of the cumulative variance in the diatom species was explained by the CCA first two axies with 89.4% the species–environment correlation. Application of the forward selection using the Monte Carlo test confirmed that the first two axes were highly significant ($P = 0.024$, $F = 3.222$, Table 3). The most effective explanatory factors were N-NO_3, EC, altitude, TN, P-PO_4, and $CaCO_3$ ($P < 0.01$).

Species distribution in relation to physical and chemical variables is given in Figure 2. The ordination of the CCA indicated that predictor variables (environmental factors) affect the distribution of diatom assemblages in the running water bodies of western Anatolia. As shown in Figure 2, nutrient-rich stations such as Dalaman stream (A8, A9, and A5) and Çavdır stream were distributed in the negative part of the first axis. On the contrary, low nutrient sampling stations (*e.g.* Kocabük, R1, Kaya, Delin, Namnam, and Karabeyyurdu creeks and Kanlı stream) were located in the opposite part. *Nitzschia angustata* (W.Smith) Grunow, *Surirella brebissonii* Krammer & Lange-Bertalot, *Diatoma moniliformis* (Kützing) D.M.Williams, and others were associated with high altitude and high concentration of nitrogen variables. A few diatoms such as *Scalptrum attenuatum* (Kützing) Kuntze and *S. ovata* var. *pinnata* (W.Smith) Hustedt were related to high water EC and P-PO_4. *C. excisa* Kützing, *Schizonema radiosum* (Kützing) Kuntze, *N. scutelloides* var. *disculus* (Schumann) Torka and *Navicula slesvicensis* Grunow in van Heurck were abundant in Kaya, Kocabük, Karabeyyurdu, and Delin creeks, which had relatively low nutrients and EC (Fig. 2).

Table 4. TIT, EQR, and EPI-D values and ecological status of the stations. Typology codes (A, flow; R, altitude; J, geology; E, slope; Y, precipitation and D, drainage).

Stations	Code	Typology	TIT	EQR	Status	EPI-D
Sarıçay creek	A2	A1R1E2Y2D1J2	2.18	0.64	Moderate	1.28
Namnam creek	A3	A2R1E1Y2D1J2	2.34	0.76	Good	1.19
Kargıcak creek	A4	A1R2E2Y2D1J2	2.11	0.61	Moderate	1.40
Dalaman stream	A5	A2R1E1Y2D2J1	2.33	0.63	Moderate	1.29
Dalaman stream	A6	A2R1E1Y2D2J2	2.28	0.77	Good	1.18
Dalaman stream	A7	A2R2E2Y2D2J2	2.48	0.71	Moderate	1.39
Dalaman stream	A8	A2R2E1Y2D2J2	2.73	0.44	Poor	1.37
Dalaman stream	A9	A2R2E1Y2D2J1	2.66	0.61	Moderate	1.88
Seki stream	A10	A2R2E2Y2D1J1	2.50	0.84	Good	1.22
Seki stream	A11	A2R2E1Y2D1J1	2.36	0.65	Moderate	1.25
Çayiçi creek	A12	A2R3E2Y2D1J1	2.21	0.8	Good	1.29
Kızılöz creek	A13	A2R3E1Y2D1J1	2.13	0.64	Moderate	1.19
Kızılöz creek	A14	A1R2E1Y2D1J1	2.28	0.69	Moderate	1.15
Çavdır stream	A15	A2R2E1Y2D1J2	2.62	0.61	Moderate	1.35
Boğluca stream	A16	A2R1E1Y2D1J1	2.56	0.83	Good	1.33
Akçay stream	A17	A2R3E2Y2D2J1	2.40	0.80	Good	1.55
Alakır stream	A18	A1R1E1Y2D1J1	2.13	0.83	Good	1.36
Eşen stream	A19	A2R2E2Y2D1J2	2.30	0.78	Good	1.10
Kanlı stream	A20	A1R1E1Y2D1J1	1.85	0.93	High	1.32
Eşen stream	A21	A2R1E1Y2D2J2	2.36	0.77	Good	1.50
Karabeyyurdu creek	BAK 12	R1D2A1J2	1.98	0.96	High	1.07
Delin creek	BAK14	A2R1E1Y2D1J2	1.88	0.98	High	1.03
Kaya creek	BAK4	A2R1E1Y2D1J1	1.53	0.99	High	1.01
Kocabük creek	BAK9	A1R1E1Y2D1J1	1.89	0.99	High	1.28
R1creek	R1	R2D2A2J2	1.84	0.91	High	1.18

Ecological status

Results of the TIT, and its related EQR and EPI-D values are presented in Table 4. The TIT values of the water bodies varied from 1.53 in Kaya Creek to 2.73 in Dalaman stream (A8). To compare the diatom indices in the water bodies, TIT and EPI-D were regressed against logTP. These relationships are shown in Fig. 3(a) and (b), respectively. Based on R^2 of the regresions, a higher proportion of variance of TIT was explained by logTP compared with that of EPI-D. Therefore, the TIT was selected for EQR calculation. The ecological status of the studied sites was evaluated using the EQR value based on the TIT. Kocabük creek had the highest EQR-TIT value (0.99), while the lowest value (0.44) was found in Dalaman stream (A8). The ecological status ranged from high to poor. Poor ecological status was observed only in Dalaman stream (A8) (Table 4), whereas high ecological status was found in Kanlı stream, Kocabük, R1, Kaya, Delin, and Karabeyyurdu creeks. Moreover, the EQR values based on the TIT also showed a negative significant correlation with logTP ($R^2 = 0.67$, $P < 0.01$) (Fig. 3(c)).

Discussion

Physicochemical variables

During the present study, Dalaman stream (A8) had higher nutrient concentrations compared to the other watercourses. This station was under pressure from human activities in Acıpayam District and several villages, and from farming processes. We suggested that the water quality of Dalaman stream could be deteriorated due to excessive addition of inorganic and organic compounds from municipal waste discharges, agricultural practices, livestock, and polluted runoff. It has already been shown that anthropogenic activities affect diatom composition and water quality (*e.g.*, Van Dam *et al.*, 1994; Reynolds *et al.*, 2002; Ács *et al.*, 2004; Bellinger *et al.*, 2006; Hlúbiková *et al.*, 2007; Padisák *et al.*, 2009; Delgado and Pardo, 2014). High population densities and the multiplicity of industrial and agricultural activities expose most hydrographic basins close to large urban centers to heavy and rising environmental impacts, especially to pollution by domestic and industrial waste residues (Mancini and Arcà, 2000; Salomoni *et al.*, 2006). In contrast, the lowest nutrient concentrations were measured in Kaya, Kocabük, R1, and Karabeyyurdu creeks. These ecosystems represented undisturbed conditions and their water beds were mainly composed of stones, gravel, and sand.

Structuring factors of diatom community composition

A total of 102 taxa mostly belonging to *Navicula* and *Cymbella* were identified in the studied area during the study period. The diatom taxa identified in this study had mostly been reported previously in other running water bodies in the USA, Italy, Tanzania, and Ireland (Potapova and Charles, 2003; Bellinger *et al.*, 2006; Della Bella *et al.*,

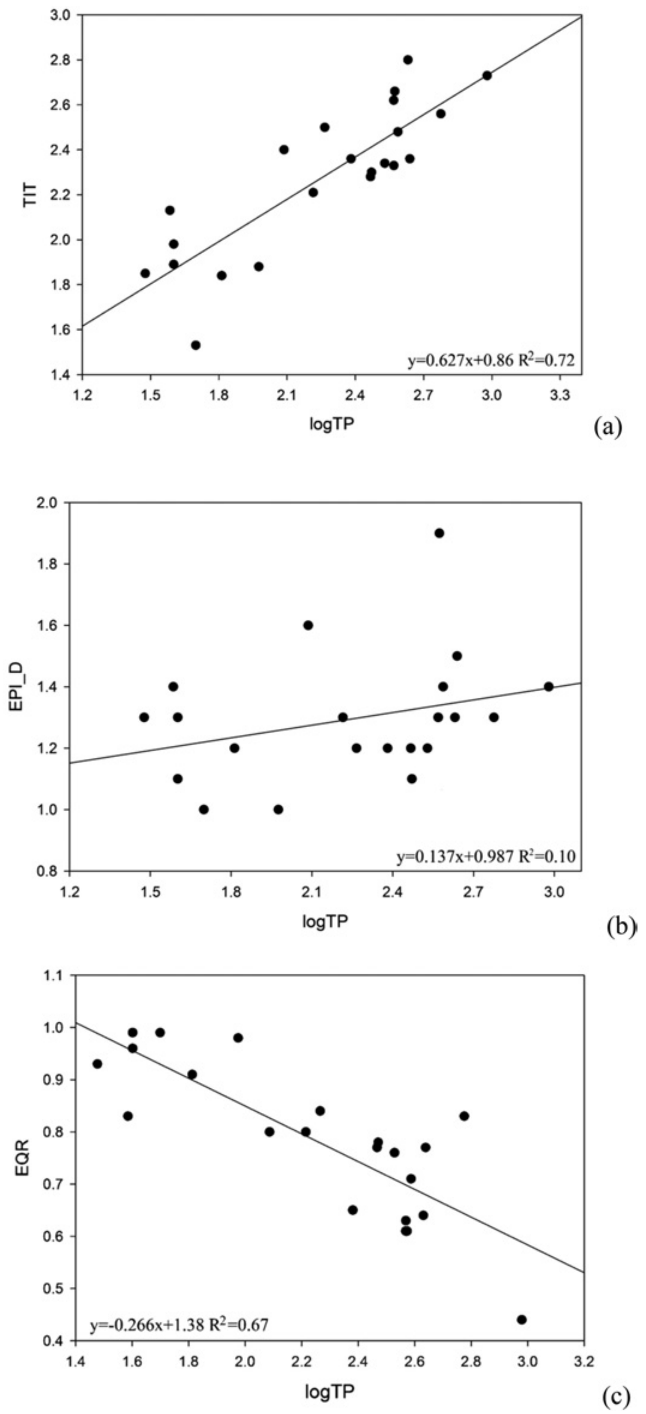

Fig. 3. Relationships between logTP and (a) TIT, (b) EPI-D, and (c) EQR at the sampling stations.

2012; O'Driscoll *et al.*, 2012). The number of species was higher than that in certain previous studies (*e.g.*, Bellinger *et al.*, 2006; O'Driscoll *et al.*, 2012).

CCA was effective in explaining diatom species–environment relationships. The results of CCA showed that *Homoeocladia umbonata* (Ehrenberg) Kuntze, *Navicula cryptocefalsa* Lange-Bertalo, and *Stephanocyclus meneghinianus* (Kützing) Skabitschevsky were associated with high nutrient concentrations (Fig. 2). *H. umbonata* (Ehrenberg) Kuntze has been indicated as a species of shallow enriched turbid waters including rivers (Reynolds *et al.*, 2002; Padisák *et al.*, 2009). *N. cryptocefalsa* Lange-Bertalo has been indicated to be a nutrient-tolerant species (Ács *et al.*, 2004) and tolerant to organic pollution and eutrophication (Salomoni *et al.*, 2006). *S. meneghinianus* (Kützing) Skabitschevsky has been considered as a pollution-tolerant species (Venkatachalapathy and Karthikeyan, 2013) and as a species typical of polluted environments (Sladeček, 1973; Kobayasi and Mayama, 1989; Van Dam *et al.*, 1994; Lobo *et al.*, 1996, 2002; Salomoni *et al.*, 2006). On the other hand, the CCA indicated that *F. capucina* Desmazières, *C. excisa* Kützing, and *Schizonema radiosum* (Kützing) Kuntze preferred low nitrogen content, especially TN, N-NO$_3$, and N-NO$_2$. *F. capucina* has been indicated as a pollution-sensitive species (Bere and Tundisi, 2011) and characteristic of good status (Kelly *et al.*, 2008). *C. excisa* Kützing has been considered to be dominant in I–II water quality (less polluted) (Gómez and Licursi, 2001). *F. capucina* Desmazières and *C. excisa* Kützing are high profile species that dislike high TSS (Marcel *et al.*, 2017) and are good competitors for nutrients (Rimet *et al.*, 2016). *S. radiosum* (Kützing) Kuntze has been reported as a sensitivity indicator species (Wang *et al.*, 2014). The occurrence of the species mentioned (pollution-tolerant or -sensitive) can reflect the integrated effect of different pressures and the water quality of the water bodies reflected by organic and inorganic pollutants.

Test of the TIT and comparison with EPI-D

The present study is the first attempt to assess the ecological status in running waters in western Anatolia using a new diatom index (TIT) according to the WFD. Relationships between TIT and EPI-D and logTP (Fig. 3(a) and (b)) indicated that TIT was better correlated to logTP ($R^2 = 0.72$, $P < 0.01$) than EPI-D ($R^2 = 0.10$). Moreover, the EQR values based on TIT also showed a significant negative correlation with logTP ($R^2 = 0.67$, $P < 0.01$). In relation to the ecological status based on the TIT, a poor status was found in Dalaman stream (A8) which had the highest nutrient contents (TN, TP; N-NO$_3$, N-NO$_2$, and P-PO$_4$). The ecological status of this station was confirmed by the presence of pollution-tolerant or eutrophication indicator species such as *H. umbonata* (Ehrenberg) Kuntze, *N. cryptocefalsa* Lange-Bertalo and *S. meneghinianus* (Kützing) Skabitschevsky. On the other hand, a high ecological status was observed in Kaya, Kocabük, Karabeyyurdu, R1, and Delin creeks. These ecosystems also had low nutrient concentrations and undisturbed hydromorphological characteristics, representing reference condition water body types as required by the WFD (Directive, 2000). High ecological status has also been reported in the lowland streams of Northern Italy (Beltrami *et al.*, 2012) using other diatom indices (IPS; CEMAGREF, 1982 and TI; Rott *et al.*, 1999) and some rivers in Ireland by O'Driscoll *et al.* (2012) using the revised TDI (Kelly *et al.*, 2008). In fact, these water bodies as well as our studied sites were located at similar altitudes (< 400 m). This situation and the occurrence of the same cosmopolitan diatom taxa observed in both our sites and above-mentioned watercourses could explain the similarity in the ecological status of the sites.

Conclusion

A total of 102 diatoms species from 25 running water bodies were identified during the present study. *F. capucina* Desmazières*, C. exisa* Kützing, *Gomphonema parvulum* Kützing, *U. ulna* (Nitzsch) Compère, and *C. placentula* Ehrenberg were commonly found in the sampling stations. The effects of multiple complex environmental variables on the diatom assmenblages were elucidated using multivariate analyses. The ecological statuses obtained for the different sites based on the trophic index correspond exactly to the state of deterioration of these water bodies due to human activities such as discharge of waste water, agriculture, and fisheries. The results of this study provide a contribution to the basin approach, and the TIT as a biological metric could be a useful tool for the assessment of running waters in Mediterranean countries.

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