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Assessment of ecological quality of coastal lagoons with a combination of phytobenthic and water quality indices

Chrysoula Christia [a], Gianmarco Giordani [b], Eva Papastergiadou [a,*]

[a] Department of Biology, University of Patras, Patras, PO 26500, Greece
[b] Department of Life Sciences, University of Parma, Via Usberti 11/a, I-43124 Parma, Italy

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ABSTRACT

Coastal lagoons are ecotones between continents and the sea. Coastal lagoons of Western Greece, subjected to different human pressures, were classified into four different types based on their hydromorphological characteristics and monitored over a three year period for their biotic and abiotic features. Six ecological indices based on water quality parameters (TSI-Chl-*a*, TSI-TP, TRIX), benthic macrophytes (E-MaQI, EEI-c) and an integrated index TWQI, were applied to assess the ecological status of studied lagoons under real conditions. The trophic status ranged from oligotrophic to hypertrophic according to the index applied. The ecological quality of transitional water ecosystems can be better assessed by using indices based on benthic macrophytes as changes in abundance and diversity of sensitive and tolerant species are the first evidence of incoming eutrophication. The multi-parametric index TWQI can be considered appropriate for the ecological assessment of these ecosystems due to its robustness and the simple application procedure.

1. Introduction

The ecological quality of coastal lagoons has been mainly assessed using different indices and metrics based on chemical and biological variables (Borja and Dauer, 2008; Munari and Mistri, 2008). Trophic status is usually evaluated with nitrogen, phosphorus and phytoplankton Chlorophyll-*a* (Carlson, 1977; Vollenweider et al., 1998; Coehlo et al., 2007). After the WFD/2000/60/EC was enforced in the European Union, biological components became key elements for assessing the ecological quality of coastal lagoons. In the last decade, a large number of studies have aimed at developing bio-indicators and indices (both macrozoobenthic and macrophytobenthic), for assessing quality and integrity of coastal lagoons: BENTIX (Simboura and Zenetos, 2002), ISD (Reizopoulou and Nicolaidou, 2007), M-AMBI (Muxika et al., 2007), EEI (Orfanidis et al., 2001), MaQI (Sfriso et al., 2009), TWQI (Giordani et al., 2009), ISS-phyto (Vadrucci et al., 2013). In highly productive ecosystems, such as coastal lagoons, the primary producers are dominated by rooted macrophytes, macroalgae or phytoplanktonic communities, which often play a negligible role being outcompeted by the benthic vegetation (Viaroli et al., 2008). In coastal lagoons human pressures such as dredging, recreation and tourism development, domestic and industrial effluents, have produced hydromorphological modifications which affect water quality. Biological components have also been subjected to human influence through fishing and aquaculture, or introduction of alien species (Borja et al., 2013). Macrophytes constitute the base of the aquatic chain and can influence the chemical element content in higher trophic levels (Viaroli et al., 2008). Furthermore they are sensitive to environmental conditions and they may respond to light penetration (Obrador and Pretus, 2010) and hydrology alterations (Christia and Papastergiadou, 2007).

Coastal lagoons have a great instability due to their location in the transitional zone between land and adjacent sea. Consequently responses of benthic and planktonic species would be influenced by many stressors, e.g. terrestrial runoff, nutrient loadings, depth and turbidity, etc. (McGlathery et al., 2007). Furthermore coastal lagoons are a heterogeneous array of habitats and ecosystem typologies due to the morphology, hydrology and salinity (McLusky and Elliot, 2007; Tagliapietra et al., 2009). Thus, the typological classification becomes a key issue for achieving a correct evaluation of the ecological status. However, a specific typological classification for transitional waters has not yet been defined and its implementation by the European countries is still under development. The differentiation of transitional waters based on their natural ecological type enhance the purpose of assigning them to a physical type and ensure that reliable comparisons of its ecological status can be

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* Corresponding author.
E-mail address: evapap@upatras.gr (E. Papastergiadou).

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made (Basset et al., 2006). Some authors have proposed typologies of transitional waters based on hydro-morphological characteristics such as morphology, tidal range, size or salinity (Basset et al., 2006; Tagliapietra and Volpi Ghirardini, 2006), while others have also included human pressures such as agricultural activities or anthropogenic nitrogen and phosphorus loads (Boix et al., 2005; Cañedo-Argüelles et al., 2012).

Based on evidence from previous studies (e.g. Giordani et al., 2009; Orfanidis et al., 2011) we hypothesize that in the early phase of eutrophication neither nutrients, Chlorophyll-*a*, nor benthic vegetation alone are sufficient for detecting changes of trophic status. A combination of both is required in order to capture slow or transient changes with long lag phases.

The objectives of the current study are: (i) to define a consistent typological classification of the transitional water ecosystems of Western Greece based mainly on hydromorphological features, (ii) to investigate the variations of physicochemical characteristics among different lagoon types, and (iii) to assess the ecological status of studied lagoons by using different ecological indices based on physicochemical and phytobenthic criteria. These lagoons can be considered as interesting case studies which may identify the main drivers of lagoon responses to increasing human impacts and the more sensitive quality elements which would be able to indicate the current ecosystem evolution.

2. Materials and methods

2.1. Description of the studied sites

More than 40% of the Greek coastal lagoon surface is located along the western coast where the strong wave action of the Ionian Sea combined with low tidal ranges, high river and precipitation loads, shaped the most important Greek aquatic ecosystems (Nicolaidou et al., 2005). This study was carried out in six coastal lagoons located along the western coast of Greece, between 39°05′N to 37°30′N and 20°48′E to 21°36′E (Fig. 1). The selected studied areas are highly diverse ecosystems, protected by national legislation and included in the Natura 2000 network as priority habitat types for conservation. A number of studies on the monitoring of Biological Quality Elements have been conducted in Greek lagoons by Simboura and Zenetos (2002), Reizopoulou and Nicolaidou (2007) on benthic invertebrates, by Orfanidis et al. (2001, 2011) on benthic macrophytes in coastal lagoons of Northern Greece and by our research group (Christia and Papastergiadou, 2007; Christia et al., 2011, 2013) in southern and Western Greece.

Rodia, Tsoukalio and Logarou are the three main natural lagoons of Amvrakikos Gulf formed by the action of Louros and Arachthos rivers. Extensive areas of salt marshes e.g. Rodia marsh, reed beds and brackish water meadows surround the lagoons. The lagoons are used for the extensive culture of various species of mullet and eels which are trapped as fry and allowed to grow naturally in the lagoon (Nicolaidou et al., 2006).

Kleisova is considered as one of the most important lagoons of Messolonghi-Aitoliko lagoon complex formed by the deposits of Acheloos and Evinos rivers (Fig. 1). It is a very shallow lagoon and the communication with the open sea is regulated by the seasonal wind regime. Fisheries and salt works are traditional and very important activities for the local community. In the late 1960s, Kleisova was artificially separated in two parts to improve the exchange of the Messolonghi sewage treatment plant effluents with the sea. Agricultural and domestic effluents flow into the lagoon through temporary streams, drainage pumping stations and channels (Avramidis et al., 2010).

Araxos is located in the northwest part of Peloponnese and is characterized by a double tombolo barrier system. It is connected with the Ionian Sea through three stable, short and narrow inlets. It receives freshwater inputs only in winter by a small draining stream Christia and Papastergiadou (2007). The lagoon is used for the semi-intensive, and recently extensive, culture of mullet (*Mugil cephalus*) and sea bream (*Sparus aurata*).

Kaiafas is a deep coastal lagoon situated in the south-western Peloponnese. In the past it was characterized by almost freshwater conditions, while in the 1960s it turned to saline conditions after the dredging of the channel that connects the lagoon with the Ionian Sea. Exchange with the sea and freshwater inputs are

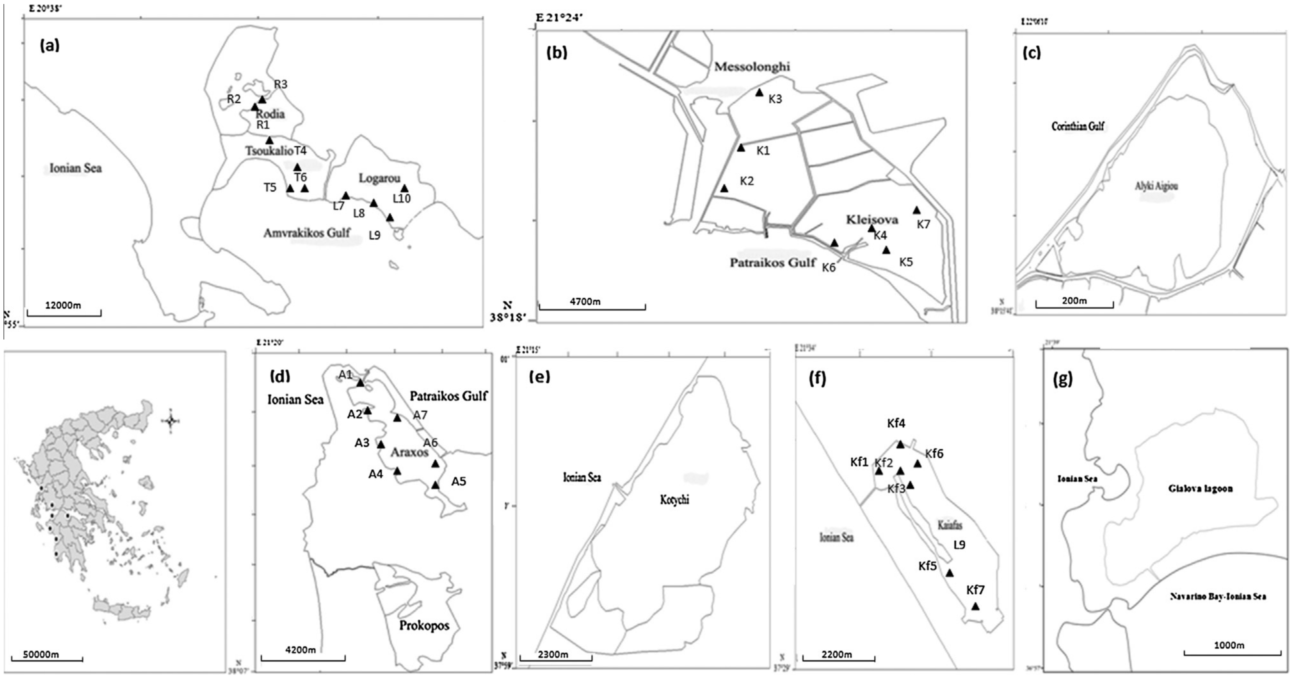


Fig. 1. Map of coastal lagoons including the sampling stations (coastal lagoons in bold) along the Western coast of Greece: (a) Rodia, Tsoukalio, Logarou, (b) Kleisova, (c) Alyki Aigiou (d) Araxos and Prokopos, (e) Kotychi, (f) Kaiafas and (g) Gialova.

irregular and mainly occurred during autumn and winter months. The lagoon receives the discharges of the wastewater treatment plant of the town of Zacharo, as well as, runoff from agricultural activities in the watershed (Bouzos and Kontopoulos, 2004; Christia et al., 2013).

In the current study, we considered also hydromorphological data of other lagoons positioned in the western part of Greece (Alyki Aigiou, Prokopos, Kotychi and Gialova) as indicated in the Supplementary Material – Table 1 in order to carry out the typological analysis on a consistent dataset. Alyki Aigiou is a small triangular area in the Gulf of Corinth. Araxos is located to the northwest of Peloponnese and connected with the Ionian Sea through three narrow inlets. Prokopos and Kotychi are brackish lagoons located in the south western part of Achaia and constitute important fishery areas. Kaiafas is a deep coastal lagoon that in the past was characterized by almost freshwater conditions. In the 1960s it turned to saline conditions after the dredging of channel that connects the lagoon with the Ionian Sea. Finally, Gialova is a lagoon located in the south western Peloponnese close to Navarino bay and in the early 1990s suffered from an oil spill (Fig. 1, Supplementary Material – Table 1). Detailed information for all studied lagoons can be found in Christia and Papastergiadou (2007) and Christia et al. (2011).

2.2. Sampling protocol

Thirty-one (31) sampling stations were homogeneously distributed in the studied lagoons of Western Greece covering the spatial heterogeneity of each particular environment (Fig. 1, Supplementary material-Table 1). Each selected sampling site was monitored at seasonal basis from spring 2005 to autumn 2007, with the exception of winter. The sampling efforts were focused during spring, summer and autumn when the responses of the systems to nutrient enrichments were more significant and growth and decay rates of macrophytobenthic species were at the highest.

At each sampling site, depth, transparency (Secchi disk), temperature, salinity, dissolved oxygen (DO) and pH was directly measured with portable equipment (WTW 340i/SET). Sub surface water samples were collected and preserved at 4 °C for laboratory analysis of nutrient [NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, Total Phosphorus (TP)] and Chlorophyll-*a* (Chl-*a*) concentrations (APHA, 1989). Water samples were filtered using 0.45 μm pore size filters for the dissolved nutrients analyses and Chl-*a* pigment extraction took place in the retained material with 90% acetone (APHA, 1989). Dissolved Inorganic Nitrogen (DIN) was calculated as the sum of the inorganic nitrogen forms.

For the monitoring of benthic macrophytes, an area of 10 m × 10 m was considered as a sampling site in each selected station. In each site three samples were randomly scraped from the bottom, in a depth range of 1–3 m, on an area of 2 m × 2 m (Selig et al., 2007). Plant species abundance was visually scored on a 5-level abundance scale ($1 < 20\%$; $2 = 21–40\%$; $3 = 31–60\%$; $4 = 61–80\%$; $5 = 81–100\%$). Macrophyte samples were placed in a plastic bag and transported to the laboratory for analysis. The samples were rinsed with water to remove sediments, identified at species level and then fixed in formalin (2%).

2.3. Indices

In this research, the following six indices (TSI-Chl-*a*, TSI-TP, TRIX, E-MaQI, EEI-c, TWQI) commonly used in lagoon trophic status assessment were applied:

(a) Water quality indices: TSI-Chl-*a* and TSI-TP (Carlson, 1977), TRIX (Vollenweider et al., 1998).

Carlson's trophic state indices compare and integrate Chlorophyll-*a* (TSI-Chl-*a*) and Total Phosphorus (TSI-TP) concentrations according to the equations: (1) $\text{TSI-Chl-}a = 9.81 * \ln(\text{Chl-}a) + 30.6$ and (2) $\text{TSI-TP} = 14.42 * \ln(\text{TP}) + 4.15$. The range of the index is from approximately 0–100, though theoretically it has no lower or upper bounds. The trophic status of the index range from oligotrophic ($\text{TSI} < 30$) to hypereutrophic ($\text{TSI} > 70$).

The TRIX index was used to characterize the trophic state of coastal marine waters. The index was calculated according to the equation: $\text{TRIX} = [\text{Log}_{10}\ (\text{Chl-}\alpha * \text{aD\%O} * \text{DIN} * \text{TP})(k)]/m$ where the parameters $k = 1.5$ and $m = 1.2$ are scale coefficients, introduced to fix the lower limit and define the extension of the Trophic Scale from 0 to 10 TRIX units. The trophic status of the index ranges from high ($\text{TRIX} < 4$), good ($4 < \text{TRIX} < 5$), moderate ($5 < \text{TRIX} < 6$) to poor ($6 < \text{TRIX} < 10$).

(b) Phytobenthic indices: E-MaQI (Sfriso et al., 2009) and EEI-c (Orfanidis et al., 2011).

The E-MaQI (Expert-Macrophyte Quality Index) index considers the macroalgal taxa present in the coastal environment and their ecological significance. The implementation of the index needs at least 20 aquatic species. All the collected taxa need to be identified at least at species level and the identification of even small epiphytes is also important. After determining all the present macroalgae, a score (0 = tolerant taxa, 1 = indifferent taxa, 2 = sensitive taxa) was associated to each taxon. However, the WFD requires that the final score range in an interval between 0 ("Bad" status) and 1 ("High" status). The ratio between the mean macroalgal scores resulting from the taxa found in the study areas and the highest value found in the "reference station" represent the Ecological Quality Ratio (EQR = mean score/highest score ratio). EQR is subdivided into five equivalent classes: "Bad" conditions: 0–0.20, "Poor" conditions: 0.21–0.40, "Moderate" conditions: 0.41–0.60, "Good" conditions: 0.61–0.80, "High" conditions: 0.81–1.0. This index was not applicable in Rodia, Tsoukalio, Logarou and Kaiafas lagoons because of the low number of species identified. The simplified version R-MaQI was not considered because the assessment of the environment is mainly qualitative and cannot be compared with other indices.

EEI-c (Ecological Evaluation Index-continuous formula) was developed by considering macrophytes as biological quality elements. It is based on the classification of macrophyte species in two Ecological State Groups (ESG) according to their morphology and life strategy traits (Littler and Littler, 1980). ESGI characterizes pristine conditions, while ESGII characterizes disturbed conditions. The sampling sites were classified in five Ecological Status Classes (bad, low, moderate, good, high) after a cross-comparison of the mean abundance values of the ESGI and II. The EEI-c values range from 2 to 10 and could be transformed into Ecological Quality Ratios from 0 to 1. The EQR is calculated according to the equation: $\text{EEI-}_{\text{CEQR}} = 1.25 * (\text{EEI-}_{\text{Cvalue}}/\text{RC}_{\text{value}}) - 0.25$ (Orfanidis et al., 2011).

EEI-c as E-MaQI can be applied both to coastal and transitional waters, but to provide acceptable results it requires the occurrence of at least 20 taxa and the greater the number of taxa is the more reliable the results are (Sfriso and Facca, 2011).

(c) Integrated index (benthic macrophytes and water quality parameters): TWQI (Giordani et al., 2009). TWQI is a quantitative, simple and easy applicable water quality index for transitional ecosystems that consider concentrations of Dissolved Oxygen, Chlorophyll-*a*, Inorganic or Total Nitrogen and Phosphorus along with the percent coverage of benthic rooted plants and floating macroalgae. These values were translated into quality values (QV) following specific relationships and weighting factors. The QVs sum of the above parameters is the TWQI values ranging from

100 (best quality) to 0 (worst quality). Ecological Status Classes are not defined in Giordani et al., 2009 but considering the quality values range of variation and the data reported in the paper we can infer that TWQI < 40 represent bad conditions, 41–50 poor, 51–60 moderate, 61–80 good and TWQI > 80 represent high conditions.

2.4. Statistics

For the typological classification of the coastal lagoons, a Principal Component Analysis (PCA) was performed using the CANOCO 4.5 software (Ter Braak and Smilauer, 2002) and the variables selected for the classification are reported in Table 1-Supplementary material. The dataset used for the typological classification of lagoons includes the studied sites of Rodia, Tsoukalio, Logarou, Kleisova, Araxos and Kaiafas and the coastal lagoons of Prokopos, Kotychi, Alyki Aigiou and Gialova which are also positioned in the western coast of Greece and described at Table 1-supplementary material. The A One way ANOVA was also conducted to test the significant differences of environmental parameters between lagoon types, while multiple *Post hoc* comparisons were performed with LSD test (SPSS V.15). Kolmogorov–Smirnov test was used to test if the scores of each index was significantly different from a normal distribution (Sig. < 0.05). The detection of significant differences between indices and lagoon types was tested with Mann Whitney test (Mann and Whitney, 1974). For each pair of significantly different parameters we calculate the effect size $r = Z/\sqrt{N}$ (Rosenthal, 1991). The criterion *r* represents a small to medium effect when is below 0.3 and a large effect if above 0.5. However, Spearman Rank correlation coefficients showed which environmental parameters were correlated significantly with the indices and contribute to different results. Additionally, the way that human pressures (Table 1-supplementary material) impacted on physicochemical parameters of each studied lagoon was shown with the implementation of PCA Ordination. Finally, in order to counteract the problem of multiple comparisons, we applied the Bonferroni correction and set the p value at 0.008.

Redundancy Analysis (RDA, CANOCO 4.5 software) was used for detecting associations between QV parameters (QV-DO, QV-Chl-*a*, QV-DIN, QV-DIP, QV-macroalgae, QV-BRP) of TWQI in different lagoon types. The RDA analysis was chosen because the Detrended Correspondence Analysis identified linear response among parameters. The matrices of explanatory variables were constructed to evaluate correlations between QV parameters and variance in lagoon type patterns. Spearman rank correlation coefficient showed the main correlation of environmental parameters with trophic indices.

3. Results

3.1. Typological classification of coastal lagoons of W. Greece

For the typological classification of lagoons, each type of lagoon was assigned considering the criteria defined by the system B of WFD 2000/60/EE and other descriptors indicated as either obligatory (latitude, longitude, tidal range, salinity) or optional (current velocity, wave exposure, mixing characteristics, turbidity, retention time in enclosed bays, mean substratum composition, mean water temperature and range) (Battaglia, 1959; Tagliapietra and Volpi Ghirardini, 2006; Basset et al., 2006). Biological aspects were not included in order to avoid circular reasoning (WFD 2000/60/EC). The tidal amplitude was similar for all lagoons (<50 cm) and was excluded from the analysis. The variables selected for the classification are reported in Table 1-Supplementary material.

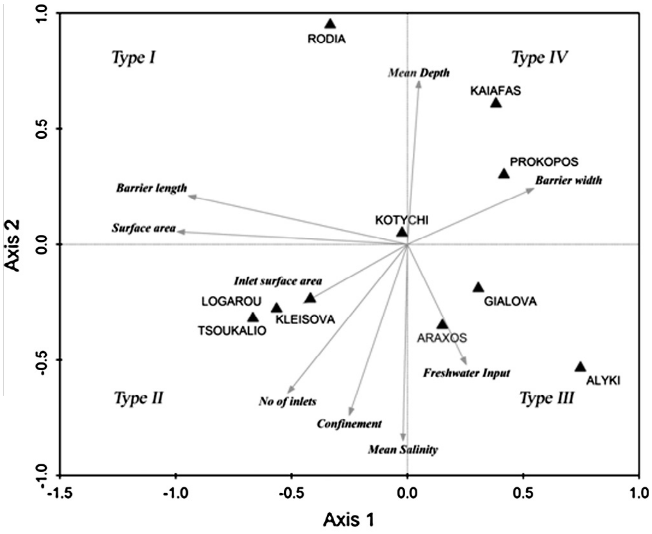
The PCA ordination showed that mean depth, confinement, lagoon surface area (width, length), mean salinity, mean depth and number of inlets were the main characteristics responsible for the classification of the studied lagoons into different lagoon types (Fig. 2).

The first two axes accounted for the 87.32% of the total variance. The first axis accounted for 70.02% of the variance and showed a gradient from large to small surface area lagoons, with low mean depth values, higher number of inlets and more saline waters from the negative to the positive part of the biplot. The second axis, on the other hand, accounted for the 17.30% and showed a gradient of deep to shallow coastal lagoons with wide lagoon barrier and small surface area.

Rodia lagoon which is positioned at the top left part of the biplot and characterized by large surface area with low mean salinity gradient is classified as Type I. Coastal lagoons positioned along the left part of the biplot, on the other hand, are characterized as large, shallow poly-haline lagoons with medium or small degree of confinement, (Tsoukalio, Logarou and Kleisova) and better communication with the marine waters. These lagoons were grouped as Type II. Additionally, Araxos, Gialova, Alyki and Kotychi lagoons which are positioned across the right part of the biplot typify small, shallow, euhaline lagoons with wide lagoon barrier and are classified at Type III. Finally, Kaiafas and Prokopos lagoons which are positioned at the top right part of the biplot, typify small, deep, meso-haline lagoons with a wide lagoon barrier and constitute the lagoon Type IV.

3.2. Environmental variables into lagoon types

A summary of arithmetic means and standard error values of physico-chemical parameters for each lagoon Type, measured during the sampling period, is reported in Table 1. Great spatial variability of almost all variables was recorded in each lagoon type as indicated by high standard error values.



	Axis 1	Axis 2
Eigenvalues	0.7002	0.1730
Surface area (Km^2)	**-0.9943**	0.0556
Mean Depth (m)	0.0508	**0.7096**
Confinement	-0.2506	**-0.7392**
Freshwater input	0.2546	**-0.5205**
Mean Salinity (‰)	-0.0191	**-0.8513**
Length barrier (Km)	**-0.9454**	0.2110
Width barrier (Km)	**0.5467**	0.2438
Inlet surf area (Km^2)	**-0.4489**	-0.2538
No of inlets	-0.5154	**-0.6432**

Fig. 2. PCA Ordination analysis based on hydromorphological characteristics of Western's Greece coastal lagoons. (PCA ordination axes in the table).

Table 1
Mean ± SE values and *F*-ratios derived from one-way ANOVA of physicochemical parameters and *Post Hoc* multiple comparisons LSD test during the survey campaign. Parameters with the same letters do not have any statistical difference for the four different lagoonal types of Western Greece.

	Type I	Type II	Type III	Type IV	*F*	Sig.
Depth (m)	1.06 ± 0.05^{a} (0.60–1.50)	0.62 ± 0.02^{bc} (0.40–1.10)	0.66 ± 0.03^{bc} (0.40–1.40)	2.85 ± 0.06^{d} (1.50–3.75)	921.26	.000
Transparency (m)	1.03 ± 0.05^{a} (0.30–1.50)	0.58 ± 0.02^{bc} 0.20–1.10	0.60 ± 0.02^{bc} (0.040–1.30)	1.98 ± 0.10^{d} (1.00–3.25)	194.34	.000
Temp (°C)	24.20 ± 0.80^{abcd} (17.70–32.50)	23.72 ± 0.34^{abc} (16.90–31.50)	24.23 ± 0.46^{abcd} (18.20–32.50)	25.59 ± 0.55^{acd} (15.30–31.10)	2.85	.038
pH	8.23 ± 0.05^{acd} (7.70–8.80)	8.47 ± 0.02^{b} (7.83–8.91)	8.28 ± 0.04^{abcd} (7.85–8.92)	8.15 ± 0.07^{abd} (7.27–9.00)	14.53	.000
DO (mg l^{-1})	7.11 ± 0.46^{abcd} (1.90–11.80)	6.99 ± 0.20^{ab} (1.90–11.90)	8.26 ± 0.37^{acd} (4.04–14.75)	8.47 ± 0.29^{abcd} (4.92–12.65)	6.99	.000
Salinity (‰)	14.09 ± 1.06^{ad} (4.10–22.40)	35.26 ± 1.12^{b} (8.80–56.10)	40.55 ± 0.53^{c} (30.90–45.10)	9.36 ± 0.32^{ad} (7.25–14.24)	152.40	.000
PO$_4$-P (μg l^{-1})	7.32 ± 1.31^{abcd} (0.82–27.20)	5.92 ± 0.50^{abcd} (0.82–32.15)	12.49 ± 1.37^{abcd} (1.91–41.52)	11.80 ± 6.18^{abcd} (1.00–274.48)	1.92	.127
TP (μg l^{-1})	107.57 ± 21.22^{abcd} (22.26–543.13)	122.68 ± 11.69^{abcd} (5.00–853.12)	119.23 ± 12.32^{abcd} (12.50–493.32)	92.91 ± 12.93^{acd} (10.00–464.89)	0.86	.461
NO$_2$-N (μg l^{-1})	21.28 ± 4.19^{abcd} (1.87–108.16)	16.45 ± 3.45^{ab} (0.001–198.31)	2.50 ± 0.35^{abc} (0.35–9.58)	28.23 ± 7.13^{abd} (0.47–153.81)	4.92	.002
NO$_3$-N (μg l^{-1})	395.97 ± 93.28^{a} (27.42–1734)	171.03 ± 24.46^{bcd} (6.39–1742)	70.15 ± 16.91^{bcd} (5.58–608.54)	103.06 ± 17.53^{bcd} (6.54–453.21)	10.66	.000
NH$_4$-N (μg l^{-1})	185.71 ± 17.66^{ad} (8.80–357.82)	74.06 ± 11.16^{bc} (0.85–848.82)	$17.77 \pm 3.14b^{c}$ (0.85–117.63)	154.31 ± 48.64^{ad} (5.50–2097.63)	8.75	.000
DIN (μg l^{-1})	602.95 ± 85.53^{a} (178.02–1826)	261.53 ± 28.90^{bd} (17.64–1858)	90.42 ± 17.55^{c} (7.95–640.95)	285.59 ± 57.36^{bd} (18.23–2276.48)	15.75	.000
CO$_3$ (mg l^{-1})	17.60 ± 1.46^{abd} (6.30–42.00)	22.02 ± 0.82^{abcd} (6.30–47.40)	24.92 ± 1.20^{bc} (9.50–56.88)	18.26 ± 1.38^{abd} (6.32–37.92)	6.71	.000
HCO$_3$ (mg l^{-1})	89.69 ± 8.05^{abcd} (12.20–219.60)	87.54 ± 3.70^{abcd} (12.20–231.80)	82.09 ± 5.44^{abcd} (12.20–201.30)	93.43 ± 5.43^{abcd} (30.50–183.0)	0.70	.555
Chl-*a* (μg l^{-1})	1.18 ± 0.22^{ab} (0.03–4.72)	1.29 ± 0.10^{ab} (0.17–6.55)	3.22 ± 0.39^{cd} (0.02–16.47)	2.49 ± 0.45^{cd} (0.03–15.36)	13.08	.000

Lagoon types I and IV were characterized by higher mean depth values than the other types. All lagoons were characterized by alkaline waters with the highest pH value measured in Type IV lagoon (9.0). During the sampling period, hypoxic events were observed only at Rodia and Tsoukalio lagoons, where dissolved oxygen concentrations decreased to values lower than 2 mg l^{-1} in summer months. Salinity was considered as one of the main parameters contributed to the classification of coastal lagoon in different types. Highly confined lagoons (Rodia-Type I) or lagoons separated from the sea by a wide barrier (Kaiafas-Type IV) showed lower salinity values. Both lagoon types II and III were characterized by higher sea water exchanges and displayed higher salinities. In particular Araxos, which is a shallow, restricted lagoon with low freshwater inputs, showed higher salinity regime comparing with Type II lagoons. Also, nitrogen compounds concentrations also differed significantly between lagoon types. Higher mean nitrate and ammonium concentrations were measured at more confined lagoons such as Rodia or Kaiafas (Table 1). Phosphate concentrations reached the highest mean values into lagoon Type II. Low Chl-*a* concentrations were measured during the whole sampling period in all lagoons, while the highest mean Chl-*a* concentrations were recorded at Types III (3.22 μg l^{-1}) and IV (2.49 μg l^{-1}).

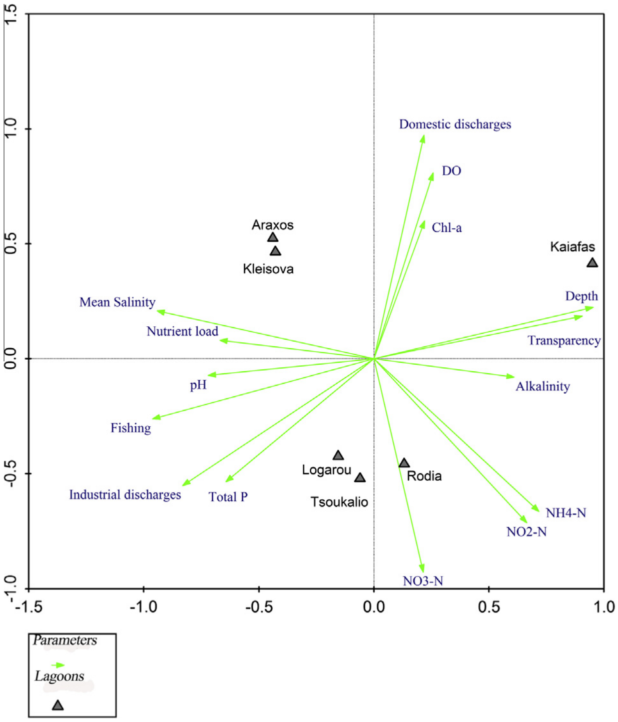


Fig. 3. PCA ordination analysis conducted in coastal lagoons of Western Greece between human pressures and physicochemical parameters. (PCA ordination axes in the table).

As shown in Fig. 3 the results of PCA ordination between human pressures and physicochemical parameters explained the 94.97% of the variance (Axis 1: 60.31%; Axis 2: 34.66%). Axis 1 showed to be positively correlated with depth (eigenvalue = 0.95), transparency (0.90) and NH$_4$-N concentrations (0.72), while a negative correlation was indicated with TP (−0.64), salinity (−0.94), pH (−0.72). Fishing (−0.96), industrial discharges (−0.83) and nutrient loads (−0.67) were negatively correlated with Axis 1. Additionally, Axis 2 was positively related with domestic discharges (0.97), DO (0.80) and Chl-*a* concentrations (0.60), while a negative relation was shown with NO$_3$-N concentrations (−0.93). Thus, in Kaiafas lagoon domestic discharges may influence Chl-*a*, pH and DO concentrations. However, high nutrient concentrations of N and P in coastal lagoons of Rodia, Tsoukalio and Logarou could be influenced by industrial discharges and nutrient loadings (Table 1-Supplementary material). Finally, intensive fishing and nutrient loadings from the adjacent agricultural lands (especially nitrates and ammonium) combined with high mean salinity values seem to affect the trophic conditions of Araxos and Kleisova lagoons.

3.3. Application of trophic and phytobenthic indices

The assessment of the trophic status of the studied lagoons ranged from oligotrophic to hyper-eutrophic levels and the ecological status classes ranged from moderate to high, depending on which index, and therefore from which ecological aspect was considered (Table 2). The Carlson's indices gave contrasting classification. Based on TSI-Chl-*a*, five lagoons resulted as oligotrophic and one as oligo-mesotrophic (Araxos), while the TSI-TP scores classified the lagoons from eutrophic to hyper-eutrophic. The water quality index TRIX did not show significant differences between lagoons, classifying their trophic status as moderate, ranging from 5.5 to 5.9. By contrast, the E-MaQI and EEI-c phytobenthic indices showed significant differences among lagoons. E-MaQI was applied only in Kleisova and Araxos lagoons where the number of species were higher than 20. The calculation of EQR indicated moderate (0.59) and poor trophic (0.34) status for Kleisova and Araxos lagoons respectively. EEI-c index showed slight differences between different types, classified them from Good to Moderate (Type III-Araxos) and Good to High (Type I-II) ecological status in contradiction with other trophic indices and E-MaQI. Furthermore, EEI-c cannot be applied in lagoon Type IV because *Potamogeton pectinatus* and *C. hispida f. corfuensis* are not listed in any ESG group. Recently, the charophyte species *Lamprothamnium papulosum* was included in the ESGI group (Orfanidis et al., 2011).

The application of TWQI index showed significant differences between lagoons characterizing their trophic status as good. The application of TWQI in Kaiafas lagoon was investigated due to the specificity of both dominant species. The benthic macrophytes of this lagoon (Type IV) differed significantly compared with other lagoon types. The dominance of the angiosperm *P. pectinatus* with the coexistence of the charophyte *Chara hispida f. corfuensis* raises difficulties in the analysis. *P. pectinatus* is a benthic rooted plant and according to TWQI high abundance of rooted plants gave high QV values. As *P. pectinatus* was a submerged species typical of mesotrophic to eutrophic waters it was included in the "floating macroalgae" group in that it has similar ecological significance. *Chara hispida f. corfuensis*, on the other hand, belongs with Chlorophyta but it is typical of low trophic conditions systems (Schubert and Blindow, 2003). In the current study, it was considered as a "benthic rooted plant" due to its morphological characteristics and ecological significance. Due to these considerations, the distinction between "floating macroalgae" species and "benthic rooted plants" in TWQI was reconsidered.

The differences between indices and lagoon types were tested with Mann – Whitney test (Sig. < 0.01). The results of the test (Table 3) indicate that TWQI index differs significantly among all lagoon types. For the phytobenthic index EEI-c significant differences were recorded between Type I and III ($U = 336.5$, $p < 0.01$) as well as between Type II and III. Furthermore, E-MaQI index also differs between Type II and III and the application of Carlson's index based on Chl-*a* (TSI-Chl-*a*) was found to differ significantly between lagoon types. Finally, TRIX and TSI-TP did not show significant differences between lagoon types.

3.4. Correlation of environmental parameters with trophic and phytobenthic indices

The results of Spearman rank correlation showed that TSI-Chl-*a* estimations were positively correlated with TP, while a negative correlation was recorded with temperature, DO and ammonia (Table 4).

This index was positively correlated with TRIX and showed a negative correlation with phytobenthic index EEI-c. Chl-*a* concentration is a key component parameter of TRIX index and its high concentrations may affect the presence and the abundance of submerged species belonging to ESGI group. TSI-TP, on the other hand, was negatively correlated with DO and positively with Chl-*a*, TSI-Chl-*a* and TRIX. E-MaQI which is based on the sensitivity of macrophyte species on anthropogenic activities was negatively correlated with transparency and phosphates. Furthermore, EEI-c was negatively affected by depth, phosphates and Chl-*a*, as these parameters were responsible for the composition of macrophytic species and high phosphorous and Chl-*a* concentrations sustain the abundance of ESGII group species. TWQI was negatively correlated with TRIX and showed a strong positive correlation with depth and transparency.

3.5. Comparison among water quality and phytobenthic indices assessments

In terms of trophic state and water quality, the application of these indices leads to a classification of the lagoons of Western

Table 2
Mean values and standard deviation of the selected trophic and phytobenthic indices were calculated into the lagoons of Western Greece during the sampling campaign. Differences between lagoons were estimated with LSD test. The same letter indicated no significant difference.

Lagoon Types	Type I	Type II			Type III	Type IV	
Lagoons	Rodia	Tsoukalio	Logarou	Kleisova	Araxos	Kaiafas	Sig.
Water quality indices							
TSIChl-*a*	27.1 ± 10.9^{abcdf}	32.1 ± 6.7^{abcdf}	31.3 ± 6.8^{abcdf}	29.2 ± 6.8^{abcdf}	38.5 ± 9.8e	31.0 ± 17.9^{abcdf}	.000
(Min–Max)	3.8–45.8	15.3–44.6	13.2–42.8	14.6–49.0	8.9–58.1	0.3–62.9	
TSITP	67.1 ± 10.8^{abcdef}	66.5 ± 17.5^{abcdef}	70.7 ± 13.0^{abcdef}	65.6 ± 13.5^{abcdef}	69.8 ± 10.0^{abcdef}	65.4 ± 13.1^{abcdef}	.293
(Min–Max)	48.9–94.7	4.2–96.9	44.6–91.1	31.3–101.5	45.8–93.6	37.4–96.2	
TRIX	5.9 ± 0.8^{abcef}	5.8 ± 1.0^{abcdef}	5.9 ± 1.0^{abce}	5.5 ± 0.6^{abdf}	5.8 ± 0.6^{abcef}	5.5 ± 1.0^{abcdef}	.070
(Min–Max)	4.4–7.1	3.8–7.4	3.8–7.3	4.2–6.7	4.4–7.0	3.8–7.6	
Phytobenthic indices							
E-MaQI				0.9 ± 0.3	0.6 ± 0.4		.000
EQR				0.59	0.34		
(Min–Max)				0.0–1.4	0.0–1.7		
EEI-c	7.5 ± 1.0^{abcd}	7.5 ± 1.0^{abcd}	7.8 ± 1.0^{abcd}	8.2 ± 1.4^{acd}	5.7 ± 2.5^{e}		.000
EEI_{CEQR}	0.68 ± 0.12	0.71 ± 0.12	0.73 ± 0.12	0.71 ± 0.24	0.47 ± 0.30		
(Min–Max)	4.8–9.0	5.9–9.8	5.4–9.7	5.4–10.0	1.0–10.0		
Multimetric index							
TWQI	73.3 ± 9.0^{ad}	66.7 ± 5.3^{bce}	62.3 ± 13.1^{bce}	73.7 ± 10.6^{ad}	66.0 ± 10.3^{bce}	77.8 ± 13.2^{f}	.000
(Min–Max)	47.0–91.0	52.0–74.0	34.0–88.0	50.0–91.0	43.0–81.0	52.7–97.3	

Table 3
Results of the Mann Whitney U tests regarding the water quality indices applied in the four different lagoonal types ($p < 0.01$).

	Type II	Type III	Type IV
Type I	$U_{TWQI} = 1132, z = -1.968, p < 0.05, r = -.17$	$U_{TWQI} = 410, z = -2.832, r = -.323$, $U_{EEI} = 336.5, z = -3.615, r = -.412$ $U_{TSIChl\text{-}a} = 279, z = -4.228, r = -.482$	$U_{TWQI} = 51, z = -6.44, r = -.76$
Type II		$U_{E\text{-}MaQI} = 1153.5, z = -5.960, r = -.50$, $U_{EEI} = 1185, z = -5.811, r = -.46$ $U_{TSIChl\text{-}a} = 1197, z = -5.765, r = -.45$	$U_{TWQI} = 129.5, z = -9.181, r = -.74$ $U_{TSIChl\text{-}a} = 1779, z = -2.631, r = -.21$
Type III			$U_{TWQI} = 19.5, z = -8.192, r = -.85$ $U_{TSIChl\text{-}a} = 789, z = -2.357, r = -.24$

Table 4
Spearman rank correlation coefficients among environmental parameters and trophic status indices applied to coastal lagoons of Western Greece.

	Water quality indices			Phytobenthic indices		Multimetric index
	TSIChl-*a*	TSITP	TRIX	E-MaQI	EEI-c	TWQI
Depth (m)					−.170*	.256**
Transparency (m)				−.143*		.343**
Temp (°C)	−.148*					
DO (mg l^{-1})	−.259**	−.173**	−.218**		.372**	
Salinity (‰)		.153*				−.163*
PO_4-P (μg l^{-1})	.228**		.267**	−.254**	−.211**	−.142*
TP (μg l^{-1})	.183**	.876**	.374**			−.199**
NO_3-N (μg l^{-1})			.241**			
NH_4-N (μg l^{-1})	−.173**					
CO_3 (mg l^{-1})			−.148*			
HCO_3 (mg l^{-1})			−.135*			
Chl-*a* (μg l^{-1})	.815**	.158*	.498**		−.208**	−.372**
TSIChl-*a*		.276**				
TRIX	.650**	.461**				
E-MaQI					.159*	
EEI-c	−.209**					
TWQI		−.167*	−.135*	−.198**	.555**	

* Correlation is significant for $p < 0.05$.
** Correlation is significant for $p < 0.01$.

Greece which have a status ranging from poor to high trophic status.

Contrasting TSI estimations based on Chl-*a* and TP concentrations were reported about all coastal lagoons of Western Greece (Table 2). According to TRIX, the trophic status of the studied lagoons correspond to a moderate level and the eutrophication mainly depends on phytoplankton community (Chl-*a*). The results of Spearman Rank correlation (Table 3) showed that TSI-TP and TRIX index did not differ significantly among lagoon types, whereas TSI-Chl-*a* showed significant difference ($p < 0.01$). However TRIX and Carlson's indices are mainly developed for coastal seas and lakes where high water depth imposes different dynamics from those in the shallow transitional waters in which benthic vegetation can be more indicative of water quality. These indices are low sensitive to changes driven by the biological components of the ecosystem and remain rather constant when the benthic vegetation changes (Giordani et al., 2009). The performance of Principal Component Analysis between lagoons and evolving parameters of TWQI (Fig. 4) accounted for 79.1% of the variance. Component 1, which explained 60.2% of the total variance, showed that the coverage of macroalgae (eigenvalue = −0.85) and benthic rooted plants (−0.90) played a significant role in the results of TWQI. Component 2 can account for only 18.9% and is correlated with DO (0.31) and DIN (−0.61).

The contribution of individual QVs to TWQI in each lagoon Type of the studied areas is shown in Fig. 5a. The lagoons with high macroalgae species abundance showed lower index values, whereas the dominance of benthic rooted plants contributed significantly to achieving high TWQI estimations. Thus, a decreasing trend of

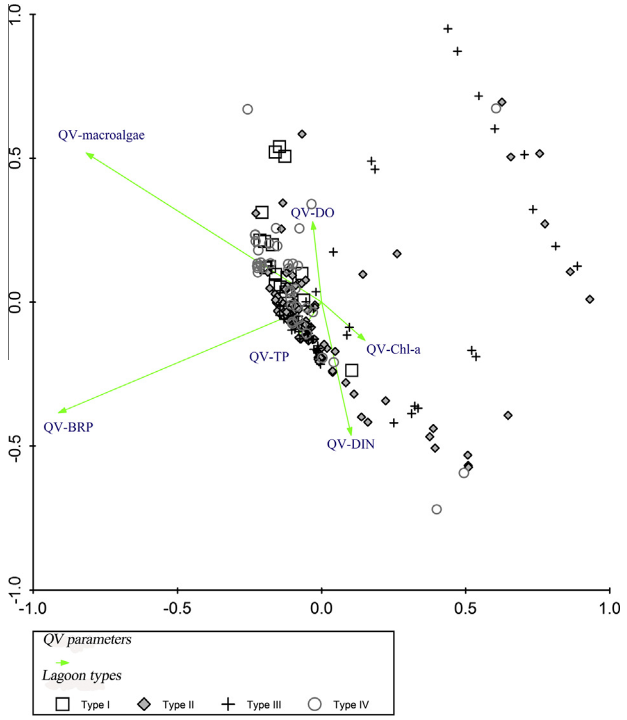


Fig. 4. Biplot of Principal Component Analysis based on the quality values (QV) of TWQI index calculated in each lagoon type. (PCA ordination axes in the table).

TWQI values was found as component 1 increased. As a consequence, coastal lagoons with higher TWQI values were positioned across the left part of the biplot such as Type IV (Kaiafas) and Type I (Rodia). In addition, some of the sampling sites belonging to Type II (Logarou) and Type III (Araxos) were positioned in the upper and bottom right parts of the biplot indicating lower TWQI values.

In the Rodia lagoon, the lower TWQI value was recorded during spring 2005 due to low DO values. In the Tsoukalio lagoon, the higher TWQI value was monitored during spring 2006 when high DO concentrations and higher coverage of benthic rooted plants were monitored. Additionally, in the Logarou lagoon the lower value of the index was measured during spring 2005 which depended on low DO concentrations and high values of DIN and macroalgae coverage. As far as Kleisova lagoon is concerned, the lower TWQI was recorded in summer 2006. Furthermore, in the Araxos lagoon the lower value of the index was calculated during spring 2006, due to high Chl-*a* concentrations and high macroalgal coverage. Finally, in the Kaiafas lagoon the higher trophic status was recorded during spring of 2007, while the lower TWQI values were reported in autumn of 2006 (Fig. 5a).

A typical case is Logarou lagoon (Type II) in which the coverage of macroalgae was high in spring/summer 2005 and summer 2006 (Fig. 6a). During these periods, TWQI estimations were lower than 60, indicating medium trophic status, while TRIX values ranged between 4 and 5 indicating a good trophic status. Furthermore, TWQI showed a gradual degradation of water quality as the macroalgal coverage increased, while there was an opposite indication in the case of TRIX. TRIX proved to be no sensitive to the alterations

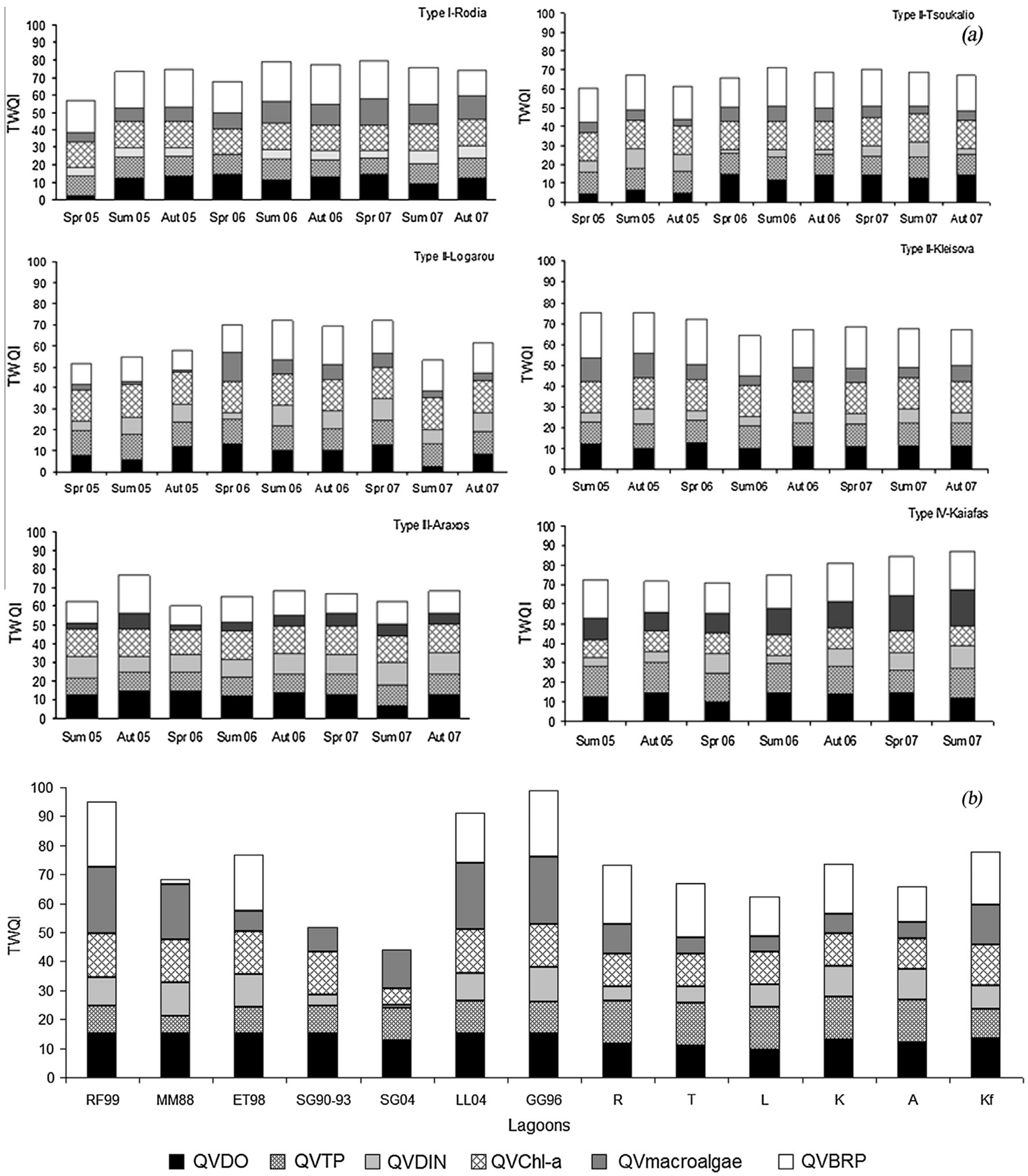


Fig. 5. (a, b) Seasonal variations of QV values of DO, Chl-*a*, DIN, DIP, Macroalgae and BRP (benthic rooted plats) applied into different lagoon types of Western Greece. Comparison of QV values of DO, Chl-*a*, DIN, DIP, Macroalgae and BRP in Sacca di Goro (SG), Lagoon of Lesina (LE), Etang du Thau (ET), Ria Formosa (RF), Mar Menor (MM) and Gulf of Gera (GG) with the studied lagoons of Rodia (R), Tsoukalio (T), Logarou (L), Kleisova (K), Araxos (A) and Kaiafas (Kf) (modified from Giordani et al. (2009)).

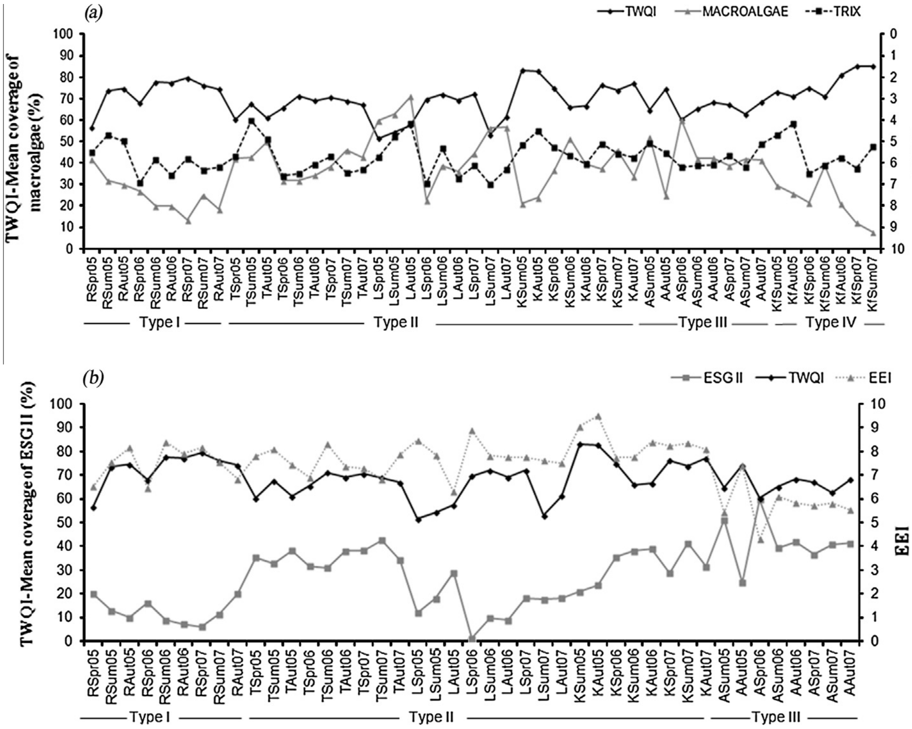


Fig. 6. (a) and (b) Seasonal evolution of macroalgae coverage (%) and Ecological Status Group (ESGII) coverage (%) in accordance to (a) TWQI and TRIX values and (b) TWQI and EEI-c during the sampling campaign in six coastal lagoons of Western Greece. The TRIX scale is reversed to have higher quality values upwards for both indices. (R = Rodia; T = Tsoukalio; L = Logarou; K = Kleisova; A = Araxos; Kf = Kaiafas).

caused to benthic elements as it focuses on water column parameters. TWQI, on the other hand was less sensitive to changes caused by nutrients and Chl-*a* seasonal variations.

Spearman rank correlation coefficient showed that EEI-c was positively correlated with TWQI, as they both consider benthic macrophytes as important parameter in the calculation (Table 4). In Fig. 6b, the relation of both indices was depicted with the mean coverage of ESGII species. When ESGII coverage was low, both indices had high values indicating a good trophic status. Furthermore, both indices were negatively correlated with PO_4-P and Chl-*a* which play a crucial role in the distribution of macroalgae.

4. Discussion

4.1. Indices applications to the identified lagoon types

Since a common typology for Mediterranean transitional water bodies does not exist, the findings of this study showed that the variability of hydromorphological characteristics among lagoons of Western Greece may contribute to their classification into different types. It is interesting to note that the four different lagoon Types (I–IV) showed a gradient in salinity ranging from mesohaline, in highly confined areas, to hypersaline, in areas more influenced by marine intrusions. Our results on the lagoon typology confirmed what was identified by Basset et al. (2006) and Tagliapietra and Volpi Ghirardini (2006), that salinity is one of the most important variables determining the composition and abundance of benthic species (Boix et al., 2005; Lucena-Moya et al., 2009). Although transitional waters are characterized as shallow ecosystems, depth limits plays a significant role in the typological classification (Type IV). Shallow depth is responsible for key features such as water turbidity, benthic vegetation, sediment biogeochemistry and nutrient stoichiometry which are of paramount importance in determining community assets and ecological successions (Zaldivar et al., 2008). According to our results, lagoon size and confinement appear to affect not only the classification of the lagoons into types but also the variability of species composition (Reizopoulou and Nicolaidou, 2004; Basset et al., 2006; Canu et al., 2012).

The values of physical, chemical and biological parameters measured in the lagoon types indicated a wide spatial gradient of water characteristics during the study period. This gradient, typical of transitional systems, was essentially due to the mixing of freshwater with seawater and anthropogenic interventions. In some cases the phytoplankton activity can strongly modify the content of nutrients, which is the cause of summer depletion of NO_3-N in many coastal lagoons. Furthermore, high salinity values may change the chemical composition of water (Gikas et al., 2009). Lagoon Type I which is characterized as mesohaline with high degree of confinement is influenced by river inputs (Christia and Papastergiadou, 2007), industrial discharges and nutrient load, showing higher nitrate concentrations (Fig. 3). This form is a major component of plant fertilizers and its concentration in transitional waters is usually linked to run off discharges (Pérez-Ruzafa et al., 2007). During summer, dissolved oxygen in the water column may decrease to hypoxic level and under these conditions sediments may act as an internal source of nutrients (e.g. ammonium and phosphates; Bonanni et al., 1992), which are known to strongly influence the composition of macrobenthic communities. Ammonia may originate from agricultural fields runoff, where irrigated crops are grown and inorganic fertilizers are frequently used (usually as ammonium nitrate). Ammonia may also originate from the decomposition of nitrogen-containing organic compounds such as decaying phytoplankton (Munari and Mistri, 2012). Phytoplankton Chl-*a* values were higher both in lagoon types III and IV. The coverage of macrophyte such as *Ulva* spp. and *P. pectinatus* in these lagoon types increases especially during dry seasons when nutrient inputs are low but decomposition rates are high, as in the case of Venice lagoon (Zaldivar et al., 2008). In addition, according to several authors (Duarte, 1995; Sfriso et al., 2003), *Ruppia cirrhosa* and some macroalgae species, such as *Ulva lactuca*, has been found to be present in conditions of high salinity and phosphate concentrations. Similarly, high biomass of *Ulvaceans* was found to have high nutrient uptake rates which led to low nutrient and Chl-*a* since these chlorophytes outcompete with phytoplankton (Viaroli et al., 2001).

The nature of any water body, irrespective of the human pressures, influences the nature of the indices and their behavior (Borja et al., 2013). Hence, the application of several indices in coastal lagoons of Western Greece showed contrasting results. In the case of TSI-Chl-*a* and TSI-TP, coastal lagoons trophic state assessment ranged from oligotrophic to hypereutrophic with some disagreements. This phenomenon is also common in other Southern European coastal lagoons such as in Foz de Almargem (Coehlo et al., 2007), Ter Vell (Badosa et al., 2008), Ricarda, Cal Tet, Ca l' Arana lagoons (Cañedo-Argüelles et al., 2012) and Kaiafas lagoon (Christia et al., 2013). In these stressed systems, low light penetration due to high turbidity is more likely to limit phytoplankton growth than N and P concentrations. In shallow ecosystems, high turbidity induced by wind-sediment re-suspension and/or prolonged flooding periods (Viaroli and Christian, 2003), may limit Chl-*a* concentration below the values expected from the nutrient availability.

With reference to the different lagoon types, their differences among the macrophyte species composition depend mainly on the salinity regime and the degree of confinement. Thus, environments with high marine influence show high species richness and vice versa (Reizopoulou and Nicolaidou, 2004; Pérez-Ruzafa et al., 2007). Species which are able to acclimate to a wide range of environmental conditions are more likely to establish in different habitat types and become more abundant, while a small number of individuals and their distribution are driven by their ability to disperse, establish and adapt to environmental conditions (Charpentier et al., 2005). Chocked lagoons (Type I) were characterized by lower number of species compared with other lagoon types (Zaldivar et al., 2008; Christia et al. unpublished data) in which angiosperms and charophytes were more common. At increasing nutrient loads, opportunistic and ephemeral species such as *Ulva*, *Gracilaria*, *Laurencia* and *Acanthophora* became more abundant inducing degraded conditions. Types II and III, on the other hand, which were less confined, were dominated by the angiosperms *R. cirrhosa* and *Cymodocea nodosa*, while sessile and perennial macroalgae like *Valonia* and *Acetabularia* were also present (Christia et al. unpublished data).

Charophytes constitutes a really important biological component of many lagoons of W. Greece but are reported only in some lagoons of Baltic Sea (Steinhardt and Selig, 2007) and Swedish coasts (Blindow, 2000). Charophytes have not been found so far in coastal lagoons of Northern Greece (e.g. Orfanidis et al., 2011), but only in temporary ponds and lakes across Greece (Langangen, 2010; Christia et al., 2011) and Cyprus (Christia et al., 2011). In the Mediterranean region they growth in shallow saline or hypersaline lakes in Spain (Conde-Alvarez et al., 2012; De Wit et al., 2013) and in typical Mediterranean ponds (del Pozo et al., 2011; Rodrigo et al., 2013) and in Veccares lagoon (Southern France) (Charpentier et al., 2005) where salinity is ca. 2–4‰. Charophytes were described as suitable bioindicators for oligotrophic and mesotrophic conditions in lakes (Stelzer et al., 2005), however, the results of our study show that charophytes may also be present in areas with high trophic level and high nutrient concentrations (Schubert and Blindow, 2003).

In transitional waters, macroalgae and angiosperms are not differentiated since they form strictly integrated patchy meadows which occupy the same bottom surface. However, the application of EEI-c in transitional waters raises some problems. The first one is the subdivision of macrophyte taxa according to their structure. In fact, some perennial species with a well structured thallus are quite indifferent to the ecological status and can even grow in polluted environments (i.e. *Cystoseira barbata*). By contrast, a very high number of filamentous, ribbon-shaped or laminar taxa (i.e. many species belonging to *Ceramium*, *Hypnea*, *Laurencia*, *Sphacelaria*) is present only in good or high-quality environments, although in coastal waters some of them can be indicative of low ecological status (Sfriso et al., 2009). In addition, when macrophyte coverage is low (ESGI and ESGII < 30%) EEI-c in many cases is not applicable, because the index, which is based on the percent macrophyte coverage of these ecological groups, would assess these environments as "Moderate". Indeed in the presence of a negligible macrophyte biomass, the ecological status can also range from "Bad" to "High", depending on the ecological state of the environment and the ecological value of the present taxa (Sfriso and Facca, 2011).

4.2. Considerations about TWQI applications

Considering the outcomes of the indices applied to coastal lagoons of Western Greece, TWQI results more adaptable and able to identify the trophic status of lagoon types (Fig. 4). The TWQI calibration conducted by Giordani et al. (2009) in six Mediterranean transitional water ecosystems was refined in this study including the environmental and biological data collected in the coastal lagoons of Western Greece (Fig. 5). An interesting aspect considered in the present TWQI application is the presence of high coverages of Charophytes in a number of sites which was evaluated as indicative of high water quality (as previously stated in the Section 3.3) and thus able to increment the index values. TWQI assessments differ among all lagoon types of Western Greece according to Spearman Rank correlation (Table 3, Fig. 5a) and the factor which plays a crucial role is the abundance of benthic macrophytes. The new index of TWQI proposed by Giordani et al. (2009) has similar water quality data requirements than TRIX but also incorporates data from benthic macrophytes. Responses of TRIX and TWQI gave contrasting assessments not only in the studied lagoon types, but also in the other systems (Giordani et al., 2009). Responses of TRIX and TWQI showed good agreement in sites without benthic macrophytes (Fig. 6a and b). However, TRIX was developed for deeper coastal waters where phytoplankton is more indicative of the ecological quality of the system than benthic macrophytes (Sfriso et al., 2009). In addition, the application of TRIX to transitional waters –firstly introduced by Vollenweider et al. (1998) to characterize the trophic conditions of Adriatic waters – could be inadequate for the peculiar environments analyzed in the present study. Therefore, the "poor" quality status assigned to some water bodies could also be the result of TRIX inadequacy for these peculiar environments, when used alone, without appropriate comparisons with other parameters (Caruso et al., 2010).

The TWQI comparisons evidenced clear differences among lagoons as the index ranged from 52 in Sacca di Goro (SG) to 99 in Gulf of Gera (GG) (Fig. 5b). Similar differences were also monitored in the Western Greek lagoons, where TWQI ranged from 62.3 to 77.8 indicating good conditions. The highest TWQI values were recorded in Gulf of Gera (99), Ria Formosa (95), Lesina (85) which conformed to high status of benthic macrophytes. The lower TWQI values were estimated for Sacca di Goro (TWQI = 42–52) which can be assessed in poor–moderate conditions due to high nutrient concentrations, phytoplankton and *Ulva* blooms and significant losses of benthic rooted vegetation following the development of human activities in the lagoon (clam farming) and in the catchment area (Giordani et al., 2009). In all the other lagoons, TWQI values were higher than 60 assessing good ecological status which was confirmed by the presence of large meadows of angiosperms as *Zostera noltii*, *R. cirrhosa* and *C. nodosa* and the charophyte *Chara hispida f. corfuensis*.

The TWQI seasonal variations in each lagoon represent the changes of the parameters that account for the ecological status assessment during the monitoring period (Fig. 5a). Differences between lagoons were higher in summer when elevated TWQI values were estimated due to the peaks in the abundance of benthic rooted plants.

The results presented in this study suggest that TWQI index may be a suitable indicator for assessing the ecological status of coastal lagoons of Western Greece and similar Mediterranean systems. In that sense, all the elements used to calculate the TWQI are sensitive to water quality, as shown by the significant relationships given in Table 3 and Fig. 4. Overall, the integration of both water quality parameters and submerged macrophytes in the TWQI index showed a better response than each of the elements separately.

5. Conclusions

The ecological evaluation of transitional water bodies is not an easy task mainly due to the large and heterogeneous data recorded in these dynamic ecosystems. The applicability and the establishment of indices have been criticized but the combined use of environmental parameters and biological data should ensure a more reliable assessment of water quality. However, there are certain common problems to all index types, including large-scale applicability, and the definition of adequate reference conditions. There is an increasing tendency to plan coastal water management over large geographical areas. It is strongly recommended that any new method of ecological status assessment should be designed and validated for large-scale application, and adapted to different areas. This is especially true when indices developed in coastal waters are transferred to transitional waters and semi-enclosed coastal systems (Blanchet et al., 2008) or vice versa (Martinez-Crego et al., 2010).

In the Mediterranean, it is still not well established which physicochemical and/or biological indicator should be used for the assessment of the ecological quality of coastal lagoons. Both macrofaunal (e.g. M-AMBI (Muxika et al., 2007) ISS (Basset et al., 2012) and macrophytobenthic indices have been extensively used for the assessment of the ecological integrity and bio-monitoring of aquatic habitats (Barbone et al., 2011; Basset et al., 2012). The sedentary nature of macrozoobenthos, together with their ubiquitous distribution and lifecycles of measurable duration allow for both long-term and short-term analyses, and they are easy to identify with already established diversity and monitoring indices. The integration of assessments based on different quality elements is an important step of ecological status assessment of coastal lagoons and is a continuous procedure needed due to the nature of these ecosystems. The multiple use and combination of different biological indices may give a more complete picture for the correct assessment of similar areas in the future. This is because they manifest a distinct response to changes in the aquatic environment, thus serving as promising indicators of hydrologic stress and aquatic ecosystem health in general.

The evaluation of the trophic state of a lagoon can be significantly different depending on the selected indicator as each one focuses on different aspects of the ecosystem (concentration of nutrients, DO, Chl-*a*, benthic and planktonic organisms). But in the list of the indicators used in the present study, the best separation among the lagoon types was presented by the indicators that consider benthic macrophytes as more representative of the trophic state of the ecosystem. TWQI, EEI-c and E-MaQI are the indices where the weight of BQE parameter is more consistent and despite the strong limitations in the application (low number of species detected, and species not included in the ecological groups or compartments) they are able to separate the lagoonal types and provide reasonable health quality assessments in the investigated lagoons. An absolute quality assessment of the lagoons does not exist and different indicators can give different information. Nevertheless, TWQI has proved to be easily applicable and able to provide consistent water quality assessments and describe their seasonal evolution. Additionally, the general approach of TWQI allows the comparison among lagoons belonging to the same or different typological classifications. The Greek lagoons considered in this study can be seen as low impacted ecosystems due to the conservation initiatives that control the few human activities which take place in the lagoons and in the relative watersheds. Despite these initiatives, the trophic state of these lagoons is increasing and the first compartment influenced by human pressures is the benthic vegetation by means of changes in species composition and reduction of high quality species coverage.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.marpolbul.2014.06.038.

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