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ORIGINAL ARTICLE

Seasonal and spatial variations of water quality, substrate and aquatic macrophytes based on side scan sonar, in an eastern Mediterranean lagoon (Kaiafas, Ionian Sea)

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Abstract Temporal and spatial variations of environmental and water quality parameters and their relations with macrobenthic flora were investigated in an eastern Mediterranean lagoon. Kaiafas is a mesohaline lagoon, which is influenced by point and diffused sources from the adjacent agricultural land and the nearby city. Water samples were analyzed for physicochemical parameters, microbial load and primary production, on a seasonal and spatial scale, while heavy metal concentrations were measured into two different sediment cores. An overall analysis of seasonal dynamics of water parameters and Chl-*a* based on trophic index TSI pointed out the lagoon as eutrophic. According to water quality parameters the lagoon was separated into two parts. The southern sector of the lagoon which was more affected by human activities showed higher nutrients, Chl-*a*, heavy metals and total Coliforms concentrations. Sediment texture and distribution of macrophytes were detected through the acoustic side scan sonar method, which proved to be a promising tool for defining and monitoring vegetation coverage of shallow lagoons. A number of distinct echo types and three different sediment types were revealed on the basis of the backscatter level and the variability of low and high backscatter areas. Furthermore, the extension and the coverage of *Potamogeton pectinatus* and *Chara hispida f. corfuensis* were successively depicted by combining the data of SSS and ground truthing samples. Meadows of *P. pectinatus* were established to the lagoon limits where high turbidity and silty substrate occurred, while *C. hispida f. corfuensis* was limited in deep and high transparent waters with sandy substrate.

Keywords Coastal lagoon · Physicochemical parameters · Sediment type · Submerged macrophytes · Trophic status

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Introduction

Coastal lagoons are aquatic ecosystems where seawater mixes with freshwater from continental origin, and also bear a resemblance to shallow lakes as they often occur as shallow water bodies separated from the sea by a barrier and usually oriented parallel to the coastline (Kjerfve 1994). Because of their unpredictability of the incoming fluxes, physicochemical characteristics vary greatly within and between annual cycles (Viaroli et al. 2008).

During the past centuries, the establishment of human civilizations and urban development on coastal areas had direct impacts on these ecosystems. The alteration of hydrological regimes and the continuous and increasing nutrient discharges from agricultural and industrial activities, have led to the eutrophication of many coastal areas in the world (Cañedo-Argüelles et al. 2012). Bottom sediments in water bodies could accumulate heavy metal loads deriving from riverine transport, atmospheric deposition, or direct anthropogenic dumping. Several studies have demonstrated that heavy metal contamination is well recorded in sediments, and a great number of studies have focused on heavy metal contamination, e.g., in alluvial fan aquifers (Petalas 2012) or coastal lagoons (Karageorgis et al. 2012; Pastorinho et al. 2012; Kharroubi et al. 2012; Papatheodorou et al. 2002). With the implementation of the Water Framework Directive (WFD, 2000/60/EC), the European Union member states are impelled to monitor the ecological quality of all their water bodies and elaborate an efficient management plan in order to solve potential problems of their transitional waters. In Habitats Directive (92/43/EE, Annex I), it was already stated that lagoons are very important and priority habitats for conservation.

Several indicators and indices have been developed to assess water quality in transitional water bodies. The most utilized indicators are based on nutrient concentrations. The ecological status of coastal lagoons is closely linked to the abundance and diversity of aquatic macrophytes (including all aquatic vascular plants, bryophytes, stoneworts, and macroalgae) as well as, to water quality characteristics. Submerged macrophytes are key structural and functional components of aquatic ecosystems as they link processes in the bottom sediments with those in the overlying water column. Moreover, the presence and abundance of the aquatic macrophytes is highly dependent on the environmental characteristics of the water body, as well as the sediment types (Stelzer et al. 2005) and the bottom relief. The depth distribution and the species composition constitute the two most important criteria that were applied to describe the influence of degradation on the macrobenthic flora of coastal waters (Selig et al. 2007). Also, Annex V of the WFD indicates the taxonomic composition and abundance of macrophytes as key elements for identifying the ecological status in transitional waters.

Because of the important role of underwater meadows in the aquatic ecosystems and their value as bioindicators (Komatsu et al. 2003; Descamp et al. 2005), it is important to assess their spatial distribution and to identify their species composition. Various techniques have been used in the past to map and monitor submerged macrophytes. Macrophyte surveys can be undertaken using a variety of methods including grapnel transects, bathyscopes, Ekman grabs and sub-aqua survey techniques (Anderson et al. 2008). Recently, remote sensing techniques, in conjunction with geographical information systems (GIS), have been valuable tools used in cartography of macrophytes (Pasqualini et al. 1998). Remote sensing techniques are divided into optical (aerial photographs, satellite images) and hydroacoustic methods (single and multi-beam echo sounders, side scan sonar) providing large synoptic assessments of spatial patterns. Hydroacoustic techniques are free of optical technique limitations (water clarity, water surface roughness, cloud cover) and are now widely used in the assessment and study of other components of lake ecosystems, providing rapid, extensive and spatially referenced data on fish, zooplankton and bottom sediments (Hoffman et al. 2002).

Hydroacoustic methods developed for macrophyte surveys include the use of horizontally aimed side scanning sonar (SSS) systems for delineating macrophyte beds (Moreno et al. 1998) and vertically aimed echo sounders for quantifying vegetation height and density (Sabol et al. 2002). Side scan sonar has been defined as an acoustic imaging device that provides wide-area, high-resolution acoustic images of the bottom relief. Under optimal conditions it generates an almost photo-realistic picture of the bottom substrate, commonly called “sonograph”, which provides information on sediment texture and bottom relief morphology. In recent times, this innovative approach has received increasing attention.

In this paper, seasonal, and spatial changes of physicochemical parameters, salinity, chlorophyll-*a*, and submerged aquatic vegetation were analysed in relation to bottom relief, heavy metal concentrations and microbial load in Kaiafas lagoon. The main physicochemical descriptors of the lagoon were monitored in order to evaluate their role in macrophyte dynamics. We suggested that the distribution and abundance of macrophytobenthic species were influenced by water chemistry and environmental parameters. Furthermore, the role of sediment geochemistry and bottom relief on the distribution of submerged vegetation was investigated. The impact of the above parameters on submerged aquatic vegetation was tested by applying an innovative and very promising hydroacoustic method (side scan sonar) combined with ground truthing surveys. Finally, we analyzed the limits of macrophytobenthic vegetation and their interactions with depth and water chemistry.

Study area

Kaiafas, is a small (200 ha) restricted lagoon with mean depth of about 3.2 m and maximum depth of 8.1 m. It is located in Western Peloponnese (N37°30′, E21°36′) and it is best known for its thermal springs and the unique coastal forest of *Pinus pinea* and *Pinus halepensis* (Fig. 1). Kaiafas lagoon is connected to the open sea through an artificially dredged channel (whose width ranges from 8 to 18 m, Fig. 1) that crosses the coastal dunes. It receives fresh water from the thermal springs and the ephemeral freshwater influxes from the small Anygros River (Poulos et al. 2012). The fresh water inflow and the water exchange with the former lake Agoulinitsa, had developed almost freshwater conditions within the Kaiafas lagoon, that turned to saline conditions after the dredging of a channel that connects the lagoon with the Ionian Sea (in the 1960s) and the artificial drainage of lake Agoulinitsa (Poulos et al. 2012).

Materials and methods

Sampling and laboratory analyses

The present work based on water sampling data collected seasonally from summer 2005 until summer 2007 (totally seven sampling campaigns). For the purposes of this study a network of seven monitoring sampling sites (Kf_1, Kf_2, Kf_3, Kf_4, Kf_5, Kf_6 and Kf_7, Fig. 1) was installed in the lagoon to monitor macrophytes and water quality parameters. The seven sampling stations were chosen on the basis of the assessed spatial variability in the environmental characteristics of the lagoon, taking into account the ecological gradient from sea to land and the differences in anthropogenic pressures. The selected sampling sites were reached as precisely as possible by boat, using GPS. Stations Kf_1, Kf_2 and Kf_3 were located to the northwestern part of the lagoon close to the artificially dredged channel that connects the lagoon with the Ionian Sea. Stations Kf_4 and Kf_6 were established to the northern part of the lagoon close to the thermal springs of Kaiafas. Additionally, station Kf_5 was positioned to the southwestern part, behind the hotel of Greek Real Estate Agency. Finally, station Kf_7 was located to the southern part of the lagoon close to the inflow of the wastewater treatment plant of the adjacent city of Zacharo.

Depth (m) and transparency (Secchi disk) were measured in order to determine the amount of light reaching the submerged species. Temperature (°C), salinity (‰), dissolved oxygen (mg/l) and pH, were measured in situ with portable equipment (WTW 340i/SET). Water samples were

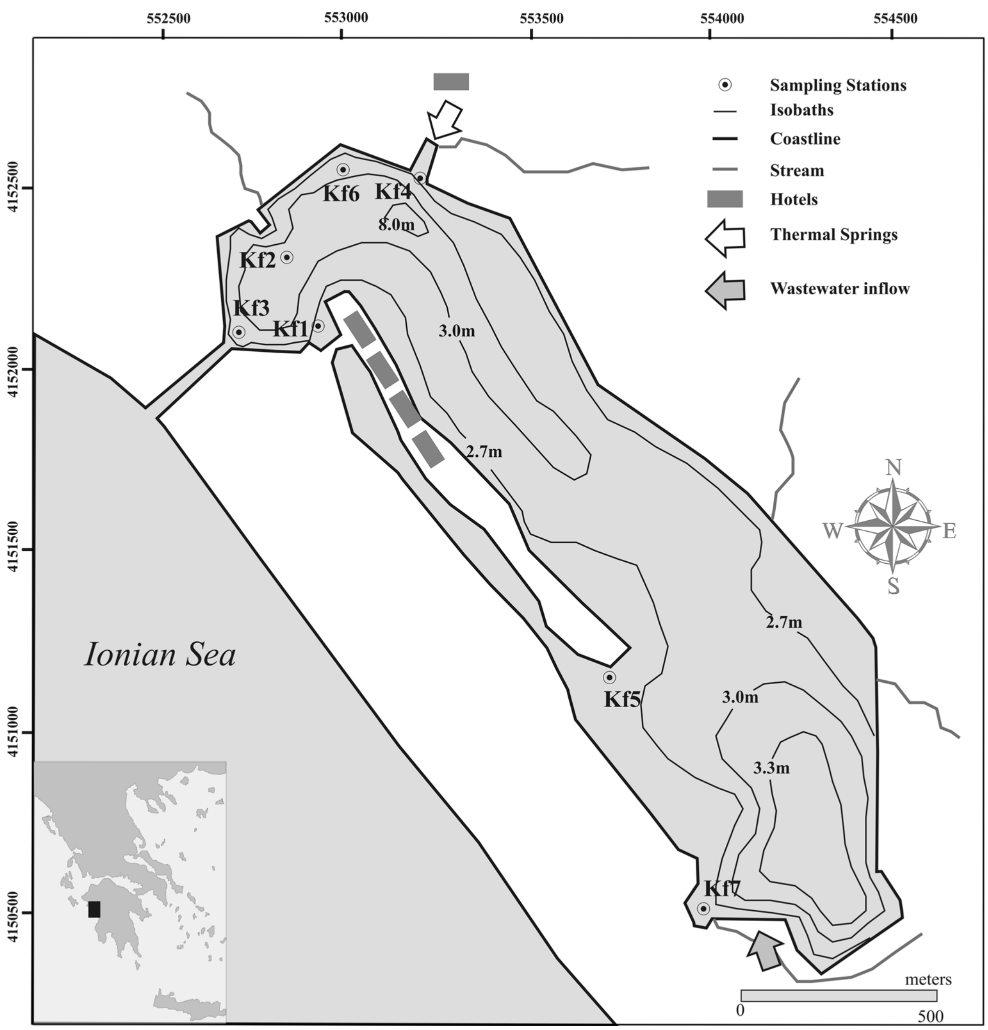

Fig. 1 A map of Kaiafas lagoon illustrating the selected sampling stations, the isobaths of the lagoon and the anthropogenic activities across the shoreline

collected between 8 and 12 a.m. from the surface water layer (30 cm depth) and preserved in cold (4 °C), dark conditions for the laboratory analyses. Water samples were filtered through a glass fibre filter of 0.45 μm GF/C. The nutrients of nitrogen and phosphorus (nitrate, nitrite, ammonia, phosphate and TP) were estimated spectrophotometrically (HITACHI UV1800) following the standard methods described in APHA (1989). Dissolved inorganic nitrogen (DIN) was determined as the sum of $N\text{-}NH_4$, $N\text{-}NO_2$ and $N\text{-}NO_3$. For the determination of Chl-*a* the spectrophotometric absorbance of 90 % acetone extracts was measured (APHA 1989).

As a sampling site for the macrophytes monitoring was considered an area of 10 m × 10 m in each sampling station (Kf1–Kf7). In each site three samples were randomly scraped from the bottom, in a depth range of 1 to 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 %).

Grain-size and geochemical analyses

Two sediment short cores of about 20–30 cm in length and 74 mm in diameter were collected once in October 2006 from the lagoon floor, using a 1 m long mini-corer. The first core was collected from the northern part of the lagoon close to thermal springs, while the second one was sampled from the southern part close to the inflow of the wastewater treatment plant. Both sediment cores were transferred to the laboratory and stored vertically at 4 °C. For the selection of the sediment sub-samples, the cores were gently cut longitudinally, measured, photographed and macroscopically described in terms of colour, texture and sedimentary structures. The sediment of the uppermost 0.5–2 cm of cores together with sediment sub-samples of 0.5–2 cm in thickness taken along the sediment core, were used for grain-size and elemental analyses. Granulometric analysis was carried out using standard sieve and pipette techniques after organic matter destruction with H_2O_2 in all sub-samples. Sediment texture and statistical grain size parameters were calculated according to Folk (1974). A quantity of each sediment sample was digested using a mixture of acids (HNO_3, $HCLO_4$, HF, HCl) (conventional method—open system) for elemental total concentration measurements (Jarvis and Jarvis 1985). Specifically, 0.1 g of each sediment sample was transferred into a Teflon tube and a mixture of concentrated HNO_3, $HClO_4$ and HF (2/1/5) was added and heated first in an open system for 3 h at 90 °C, then 3 h at 130 °C, 10 h at 190 °C and finally 1 h at 90 °C. After cooling at room temperature, 2 mL of 4 M HCL was added and heated for 1:30 h at 70 °C. Again, after cooling at room temperature, 8 mL of 0.3 M HCL were added. Finally, the digests were filtered using the Millipore polycarbonate 0.45 μm filters and diluted up to 10 mL with ultra pure water and kept at 4 °C for subsequent elemental analysis. The elemental analyses in the solutions were carried out by a Perkin–Elmer ELAN 6100 Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The organic carbon content (C_{org}, %) was determined by oxidation of the samples according to Gaudette et al. (1974).

For this study, we compiled published data from Bouzos and Kontopoulos (2004), based on the substrate surface sediments of the lagoon, which consists of granulometry, organic carbon, and calcium carbonate percentage of the surface sediments of the lagoon. Therefore, the surface sediments of the lagoon were distinguished in Sediment Types on the basis of sand–silt–clay percentages, organic carbon content and $CaCO_3$ percentage.

Microbial community analysis in the water column

The pour plate procedure was used to evaluate the microbial pollution of water sample collected from the surface layer during spring months of 2007. Standard Pour Plate (SPP) procedure proved to be the most sensitive of the methods used since microbial counts were observed at all sampling periods (including 0 h), but a 24 h period was required for the growth of viable cells. SPPs were made at each sampling by placing duplicate 1.0 and 0.1 mL portions of the undiluted DSS solution and four 10-fold dilutions (10-1 through 10-4) of the undiluted test D5S into sterile plastic Petri plates. Trypticase soy agar cooled to 48 °C was added to each plate. The contents were then mixed and allowed to solidify. Pour plates were incubated for 24–48 h at 35–37 °C, and CFU (Colony Forming Unit) per millilitre were determined (Anderson et al. 1986).

Remote sensing and ground truthing survey

The remote sensing survey of the Kaiafas lagoon was organized into two phases. First, a systematic survey of the lagoon floor was carried out using a side scan sonar technique. The second phase consisted of biological sampling based on the results of the first phase. During the second phase direct methods were adopted in order to produce the most accurate information regarding seabed coverage and thus, to validate the side scan sonar interpretation. This methodological approach provides a cost-effective tool to rapidly survey areas. It should be noticed that the side scan sonar survey and the targeted ground truthing samplings were focused on the deepest part (>2.5 m) of the lagoon, whilst the monitoring stations (Kf_1–Kf_7) were restricted to shallower waters (<2.5 m).

For the surveillance of Kaiafas lagoon was equipped an Edgetech 272 TD tow fish with a transducer, which was able to emit an acoustic signal at two frequencies of 100 and 500 kHz. The total surveying coverage was spanned about 0.4 km in a southwestern—northeastern direction and about 2.3 km from northwest to southeast. Five (5) navigation lines ran parallel to the shore of Kaiafas lagoon in a northwest to southeast direction. The lines ran 15 m apart, to allow side scan sonar sonographs to overlap. Triton Map (Triton Imaging Inc) software was used for the generation of the sea floor mosaics, which were performed at 0.5 m resolution. The SSS mosaics were exported as GeoTIFF files and then were utilized in Arc View GIS 9.2.

For the recording of the acoustic data a digital unit model Edgetech 4100 P topside was used. The acquisition software of 4100 P topside recording unit applied the geocoding of SSS digital records, using navigation and vessel speed data supplied from the GPS system model MAGELAN NAV 6500. The 100 kHz signal was considered as an effective frequency for the selection of a wide spectrum of acoustic faces indicative of a variety of seafloor texture, bed forms and particularly biological formations (Georgiadis et al. 2009).

In the Kaiafas lagoon survey, high backscatter was represented by light tones and low backscatter by dark tones, on the sonographs. No seafloor classification system has been used for the interpretation of the SSS data and the classification of lagoon floor in echo types. A number of echo types were recognised on the SSS mosaic on the basis of (1) the backscatter level, (2) the homogeneity of the backscatter, (3) the variability of low and high backscatter areas (patchy acoustic pattern) and (4) the configuration of their limits. It should be noted that the submerged aquatic meadows were distinguished by strong backscatter, which was significantly higher than the surrounding sand-covered lagoon floor. The aerial extent of each echo type was estimated using the Arc View GIS 9.2.

Based on the SSS information and the classification of SSS data in distinct echo types, ground truth sites were selected. Three (3) ground truth sites were chosen within the echo type ET6, two (2) sites from echo types ET1, ET2 and ET4 and one site from echo types ET1a and ET3. The echo type ET5 was not successfully sampled due to high water depth (6–8 m). Finally, eleven (11) ground truth sites were distributed relatively evenly through the study area.

Data processing and statistical analyses

A multivariate analysis of variance was run with season, year and sampling station as independent factors. The *Post Hoc* LSD test was performed to compare all different combinations of environmental parameters in Kaiafas lagoon during the sampling period. The tested variables were first being Log $(x + 1)$ transformed to fulfil normality and homoscedasticity assumptions (Zar 1999). In order to test for correlations between the parameters measured, the bivariate correlations procedure (Spearman Rank correlation coefficient) was used.

One trophic state index was determined, the classical TSI of Carlson (1977), that compares Chlorophyll-*a*, Total Phosphorus, and Secchi depth transparency. TSI index for three different quality variables was calculated according to the equations: (a) TSI Chl-$a = 9.81 \ln (\text{Chl-}a) + 30.6$; (b) TSI $\text{TP} = 14.42 \ln(\text{TP}) + 4.15$; (c) TSI $\text{SD} = 60–14.41 \ln(\text{SD})$. The range of the index was from approximately 0 to 100, although theoretically it has no lower or upper bounds. Finally, Carlson's TSI index was calculated as the average value of TSI Chl-*a*, TSI TP, and TSI SD.

A Principal Component Analysis (PCA) was performed on log transformed, centered and standardized data, using CANOCO 4.5 software (ter Braak and Smilauer 2002), in order to evaluate the seasonal and annual variability of the physicochemical parameters, to understand the relationships among the variables, and to analyze which of them were explaining the higher percentage of total variance. The eigenvalue showed the amount of variance accounted for the observed variables. Percentage of variance presented the percent accounted for by each specific variable, relative to the total variance in all the variables.

A Redundant Analysis (RDA) was conducted to analyze the relationship between aquatic macrophytes and environmental variables. The selection of the type of analysis was determined according to the length of the data gradient after a preliminary Detrended Analysis. The selection of explanatory variables was made using CANOCO 4.5 and the variables with a $p < 0.5$ of the Monte Carlo Permutation Test and variation inflation factors (VIF) values lower than 20 were retained (ter Braak and Smilauer 2002). The significance of the first axis and all the axes of RDA was tested with permutation tests.

Results

Water quality monitoring-trophic conditions

Kaiafas is a mesohaline lagoon where salinity ranged from 6.9–14.2 ‰ (Fig. 2). During the monitoring period there were not recorded anoxic crises, the water temperature followed the regular seasonal cycle with maxima during summer and minima during autumn, while the lagoon water was alkaline with pH ranged from 7.27 to 9.00 (Supplementary material-Table 1).

Concentrations of ortho-phosphates ranged below 10 μg/L during the whole monitoring period, while the

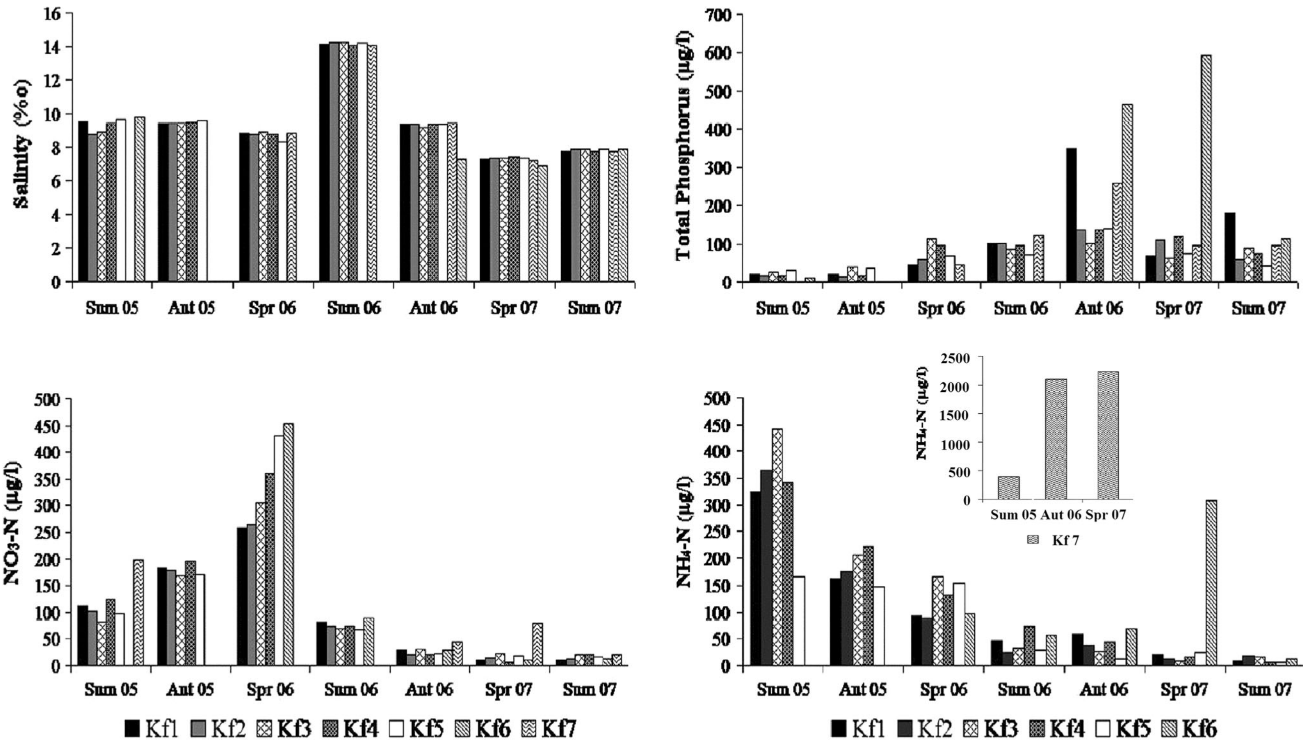

Fig. 2 Mean values of salinity (‰), TP, NO_3-N and NH_4-N concentrations (μg/L) measured at the seven sampling sites of Kaiafas lagoon during the monitoring period from summer 2005 to summer 2007

maximum value (561.3 μg/L) was measured at station Kf_7 during spring 2007. However, the mean value of TP was lower than 100 μg/L at most of the surveyed sites (Fig. 2), showing significant differences among years (Supplementary material-Table 1). A strong positive correlation of PO_4-P and TP with Chl-*a* indicated (Supplementary material-Table 2) that phosphorus could be a limiting factor for phytoplankton growth.

During the survey period nitrate concentrations showed heterogeneous spatial and temporal behavior on a seasonal and annual scale (Supplementary material-Table 1). Values higher than 200 μg/L were common during spring of 2006, while lower concentrations were measured during the rest of the monitoring period (Fig. 2). Furthermore, a sequence of high ammonium concentrations at station Kf_7 during autumn months of 2006 (2,097.7 μg/L) and spring months of 2007 (2,233.3 μg/L) was recorded, probably due to discharge of sewage from the wastewater treatment plant of the Zacharo city (Fig. 2).

Mean values of Chl-*a* concentration showed significant difference on a seasonal and annual scale (Supplementary material-Table 1). The mean annual value of Chl-*a* was always lower than 0.05 mg/m^3 at all surveyed stations during 2005. The following years of 2006 and 2007, Chl-α mean concentrations showed an increasing trend from 3.27 to 5.04 mg/m^3 respectively. High Chl-α values were measured during autumn of 2006 (15.36 mg/m^3) and spring of 2007 (26.84 mg/m^3) at Kf_7 station. The values were generally higher at the southern part of the lagoon, rather than at the northern.

The MANOVA (SPSS 15) analysis and the *Post Hoc* multiple comparisons LSD test conducted during the monitoring period revealed that environmental parameters such as the ratio of transparency to depth, TP, nitrite and nitrate concentrations and Chl-*a* showed statistically important differences among years and seasons (Supplementary material-Table 1). The significance of interactions between the three factors: season, year, station was also considered. More specifically, the interaction between season and station indicated significant ($p < 0.05$) effects for DO, PO_4-P, TP, NH_4-N and Chl-*a*. However, the interaction between season and year showed a significant effect for the ratio transparency to depth, PO_4-P, NO_3-N and NO_2-N, while the interaction between station and year affected significantly only temperature, DO and salinity parameters.

The results of Spearman Rank correlation coefficient (Supplementary material-Table 2) revealed the complex relations between the parameters and considered them as important components in order to understand the system as a filter, able to modulate changes in nutrient load. This study highlighted significant positive correlation for Chl-*a* with TP and DO ($p < 0.01$), while negative correlation was recorded with temperature ($p < 0.05$) and salinity ($p < 0.01$). Total P, on the other hand, showed significant negative correlation with temperature and nitrogen nutrients (NO_2-N ($p < 0.05$), NO_3-N and NH_4-N ($p < 0.01$)), while DO showed to be negatively correlated with temperature, salinity and PO_4-P ($p < 0.01$).

The trophic state index of Carlson determined with Chl-*a* presented lower values comparing with total phosphorus and Secchi depth indices (Fig. 3a). TSI Chl-*a* index showed minimum values on summer 2005 and maximum on spring 2007 (62.9) corresponding to oligotrophic and eutrophic conditions respectively. The majority of the TSI Chl-*a* values indicated oligotrophy to oligo-mesotrophy (72.7 %) and only 27.3 % corresponded to mesotrophic or eutrophic conditions. Moreover, TSI TP depicted oligo-mesotrophic and hypereutrophic systems. Nevertheless, for most samples (40.9 %) TSI TP values suggested hypertrophic conditions (Fig. 3b). The range of TSI SD (Fig. 3c) values in Kaiafas lagoon characterized corresponded to mesotrophic and hypertrophic conditions (72.7 %). Finally, the trophic condition of the lagoon according the total TSI index was characterized as eutrophic during the whole sampling period (Fig. 3d).

PCA ordination

PCA ordination presented a two-dimensional correlation biplot of the most important environmental parameters measured in Kaiafas lagoon during the sampling period (season-year) at the seven different sampling sites (Fig. 4a and b). The eigenvalues for PCA axes 1 and 2 captured the 57.7 % of the total variance of the data. Axis 1 showed to be highly correlated with pH (eigenvalue: 0.80), ratio depth to transparency (0.64) and Chl-*a* (0.48). However, Axis 2 was highly correlated with NO_2-N, NO_3-N, NH_4-N concentrations (0.83; 0.85; 0.69), temperature (0.68), salinity (0.51), alkalinity (0.56), TP (0.50) and PO_4-P (0.48).

The ordination biplot (Fig. 4a and b) illustrated that the horizontal axis could be informally interpreted as a gradient of the ratio transparency to depth, pH, TP and Chl-*a* showing a decreasing trend from right to left. The vertical axis, on the other hand, could be interpreted as a gradient of PO_4-P, HCO_3^-, nutrients of nitrogen, salinity and temperature. According to the PCA biplot, summer months were positioned at the bottom part of the graph indicating thus, highly positive correlation with salinity and temperature gradient. Spring and autumn months, on the contrary, were arranged at the top part of the diagram. The inter annual differences depicted in Fig. 4b showed that sampling sites of 2005 which situated to the right part of the biplot, close to axis 1, were positively correlated to nitrogen species. The sampling sites of the second monitoring period (2006) were scattered through the biplot, while those of 2007 were positioned to the left part of the biplot indicating higher Chl-*a* and TP values. Some

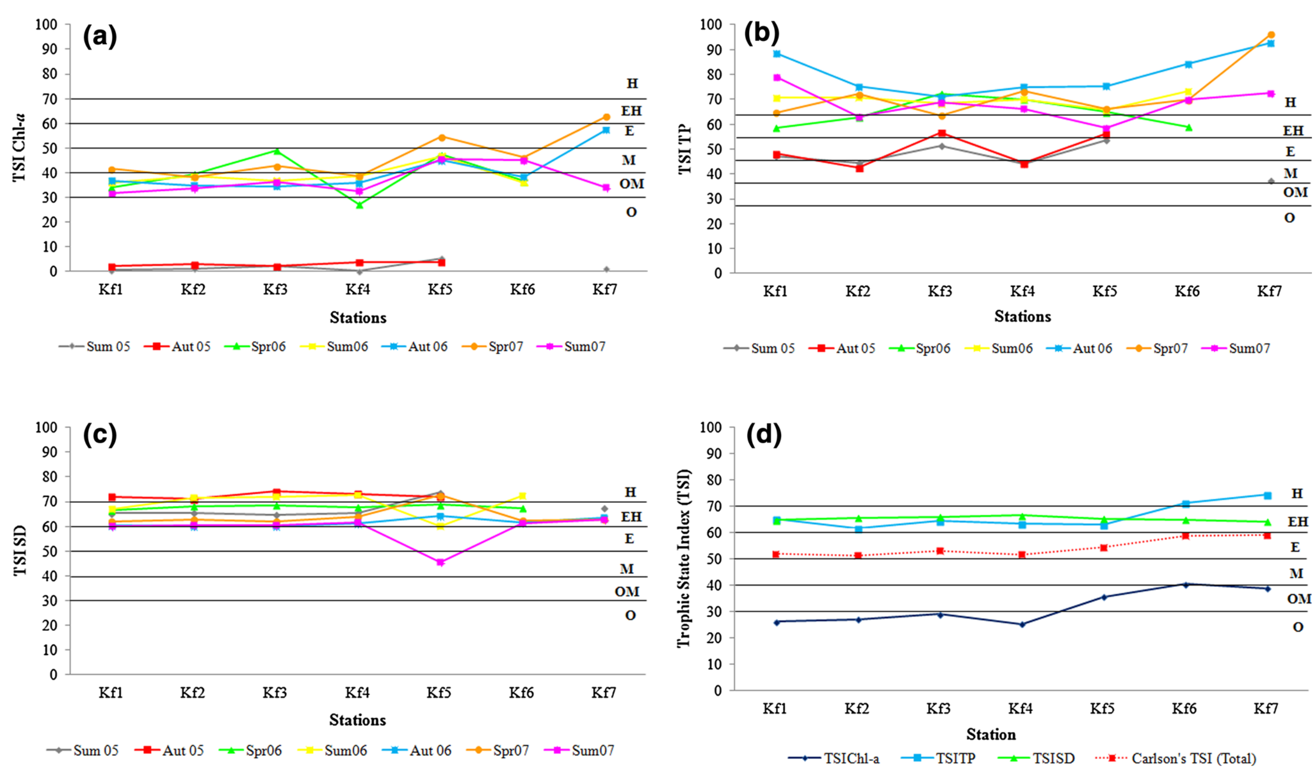

Fig. 3 a, b, c, d Seasonal variation of trophic state indices TSI with Chl-*a*, Total Phosphorus (TP), Secchi Depth (SD) and Carlson's TSI Total calculated into the seven sampling sites of Kaiafas lagoon during the monitoring period from summer 2005 to summer 2007. (*O* oligotrophic, *OM* oligo-mesotrophic, *M* mesotrophic, *E* eutrophic, *EH* eutrophic to hypereutrophic, *H* hypereutrophic

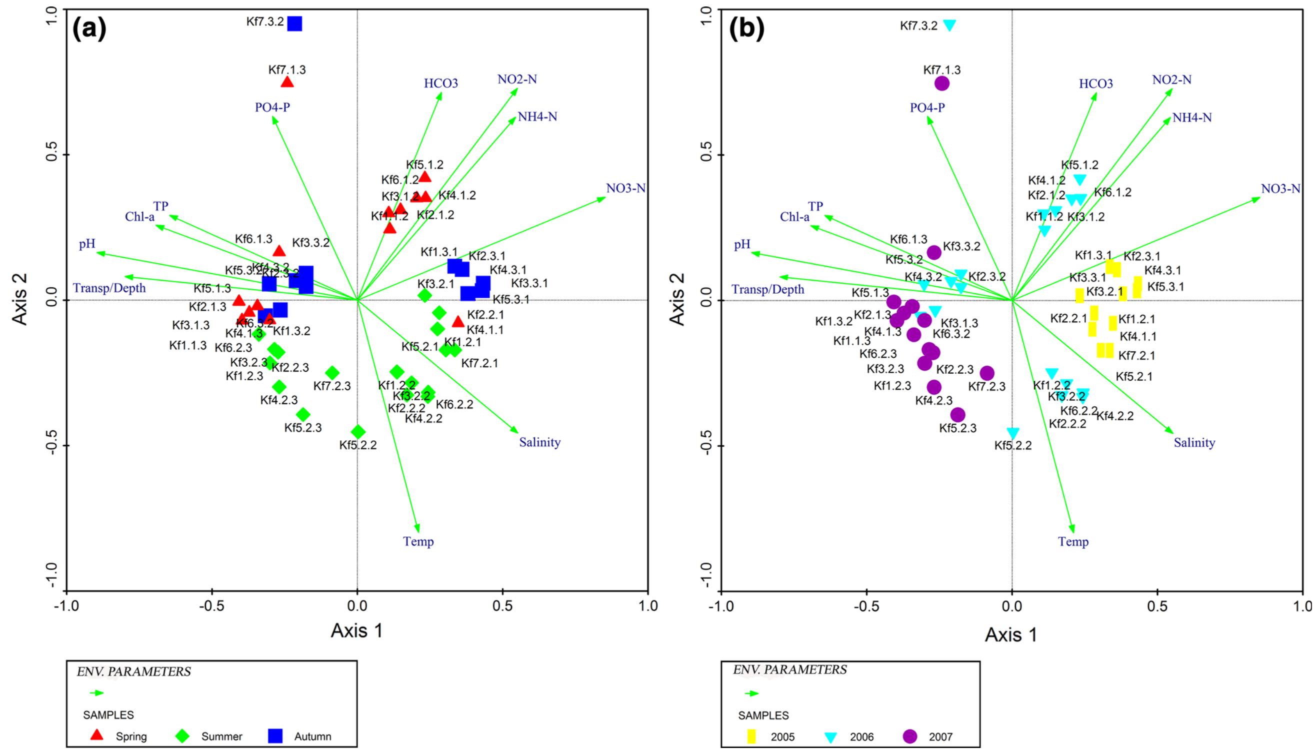

Fig. 4 a, b PCA ordination biplot presented the most important environmental parameters measured in Kaiafas lagoon during the sampling period on a seasonal and annual scale at the seven different sampling sites. The first number represents the station, the second number the season (1, spring; 2, summer; 3 autumn) and the third number the year of the survey (1, 2005; 2, 2006; 3, 2007)

environmental variables were close to each other on the ordination diagram, due to high correlation coefficients between them.

Lithology and geochemistry of the substrate and microbial community of the water column

Kaiafas lagoon surface sediments were generally fine-grained with high percentages of fine sand contents. The sediments ranged from sandy mud to muddy sand with the most of the lagoon substrate covered by sandy mud. Elevated values of C_{org} (1.77–6.10 %) were measured all over the lagoon sediments but higher values (5–6 %) were found in the eastern-southeastern part of the lagoon, adjacent to the marsh zone. The percentage of $CaCO_3$ in the surface sediments of the lagoon ranged from 22.18 to 67.21 %. The northern part of the lagoon was characterized by lower values of $CaCO_3$ (20–40 %) compared to those of the southern part.

The surface sediments were distinguished in three sediment types on the basis of the sand–silt–clay percentages, organic carbon content and $CaCO_3$ percentage (Table 1).

Sand content over 50 % (sediment Type I) predominated in the northern and southwestern part of the lagoon, whilst clay and silt contents (sediment Type II) prevailed in the northeastern and the southern part of the lagoon. Sandy mud (sediment Type III) covered an extended part of the lagoon floor and predominated in the central and south-eastern part, adjacent to the marsh zone.

Table 1 Granulometric and geochemical characteristics of Sediment Types distinguished in the Kaiafas lagoon based on previous (Bouzos and Kontopoulos 2004) and current sedimentological surveys

	Sand (%)	Silt (%)	Clay (%)	Corg (%)	$CaCO_3$
Sediment Type I	69.1	14.6	16.3	2.8	40.5
Sediment Type II	20.5	43.4	36.0	4.35	42.3
Sediment Type III	41.1	32.4	26.5	4.0	50.1

Vertical profiles of heavy metal concentrations in two sediment cores collected from the northern and southern end of the lagoon reflected the depositional/post-depositional conditions and anthropogenic threats dominating in certain areas of the lagoon. Two opposite down-core elemental trends were recorded in the lagoon. A down-core increase of the heavy metals was found in the core from the northern part (Core 1) while, an opposite and more pronounced trend, was found in core from the southern part of the lagoon (Core 2) (Table 2).

Particularly, in the northern part of the lagoon the uppermost 10 cm of dark gray mud was characterized by lower heavy metals compared to those measured in the

Table 2 Trace elements concentrations (Co, Cr, Cu, Mn, Pb, Zn, Ni in ppm) of sediment samples collected from the northern and southern part of Kaiafas lagoon during the sampling period

	Core (northen part)					Core (southern part)				
	0–3 cm	3–5	5–7	7–9	29–31	0–0.5 cm	0.5–1.0	1–2	2–3	14–16
Co	10.1	9.5	7.6	14.4	12.5	4.9	7.5	5.6	7.3	4.1
Cr	38.0	57.7	27.5	54.7	71.3	32.3	65.5	61.5	29.4	23.6
Cu	20.4	22.5	17.8	21.8	29.4	11.0	17.6	13.4	17.6	10.1
Mn	261.5	293.7	249.0	283.0	437.9	401.7	392.9	381.5	332.6	303.2
Pb	16.0	17.1	13.7	17.6	18.4	8.5	12.2	9.9	12.2	8.6
Zn	18.7	52.0	4.6	51.3	58.7	8.9	33.5	18.7	10.9	–
Ni	56.5	63.6	49.8	61.4	83.7	32.0	50.0	37.5	51.1	26.9

lower part of the sediment core (Table 2). On the contrary, in the southern part of the lagoon, a surface layer about 3 cm thick of gray muddy sand with elevated heavy metals concentration overlaid a gray mud with significantly lower heavy metal concentrations (Table 2).

Enrichment in Cr, Cu and Pb in the upper portion of the sediment core in the southern part of the lagoon was related to sediment contamination as a consequence of the entering of the wastewater in the lagoon from the neighbouring city of Zacharo.

The microbial pollution was generally not significant during the whole period. The higher Total Coliforms concentration was measured during spring of 2007 at Kf_7 station (38.800 CFU/mL after a 100 times of rarefaction) indicating the malfunction of the wastewater treatment plant of Zacharo city.

Aquatic macrophytes

Kaiafas lagoon was dominated by the angiosperm *Potamogeton pectinatus* and the endangered charophyte *Chara hispida f. corfuensis*. The latter is reported as a new species for Greece known only from the coasts of Corfu and Sifnos Islands and is included in the Red List of Charophytes for the Balkans (Bacinska Lakes, Croatia), classified as critically endangered globally according to IUCN Threat Categories and Criteria (Christia et al. 2011).

A Redundancy Analysis (RDA) (Fig. 5) was conducted environmental parameters and both aquatic species. The number of the explanatory parameters included in the analysis was reduced after a forward selection based on the Variance of Inflation Factor and the statistical significance of each variable was judged by a Monte-Carlo permutation test. Value of Inflation Factor greater than 20 indicated a strong effect of collinearity and the variable was omitted from the analysis (ter Braak and Smilauer 2002). The explanatory variables (Chl-α, TP, PO_4-P, NH_4-N, depth, alkalinity, salinity, DO, pH, sediment Types I, II, and III) retained by the forward procedure and low Variance of Inflation Factor (VIF). Axis 1 of the analysis explained the 60.3 % and both permutation tests were highly significant ($p < 0.05$).

The RDA revealed that *C. hispida f. corfuensis* which is located to the left top side of the biplot indicated high preference to the sediment Type I dominated by maximum average of sand and minimum average of clay and silt. The biplot also indicated the strong positive effect of the charophyte meadows on water transparency and the preference to deepest waters. Furthermore, several other components such as dissolved oxygen, pH and salinity seem to have positive correlation to the distribution of the charophyte. On the contrary, *P. pectinatus* which is positioned to the top right side of the biplot indicated high preference to the sediment Type II which is characterized by minimum average of sand content and maximum average of clay and silt (Table 3). The presence of *P. pectinatus* was related less strongly to changes in depth and transparency than *Chara* species. The nutrient status of the lagoon seems to affect the presence of charophytes which was negatively related to nutrient concentrations of N and P.

Acoustic mapping of bottom relief and macrophytes beds

The SSS survey was used to illustrate the distribution and abundance of submerged macrophytes in the deepest parts of the lagoon. With the application of this hydroacoustic technique over the 50 % of the lagoon substrate/bottom was systematically mapped.

The acoustic signal indicated that the entire insonified area was covered by submerged plants. However, side scan sonographs were ascribed to seven echo types based on the consideration of their backscatter intensity, homogeneity and “patchy pattern”, their extent and the configuration of their limits (Figs. 6 and 7). Echo type 1 (ET1) presented areas of high reflectivity confined by distinct and sharp limits. The presence of acoustic shadow zones at the ET1 suggested elevated areas often up to the surface of the

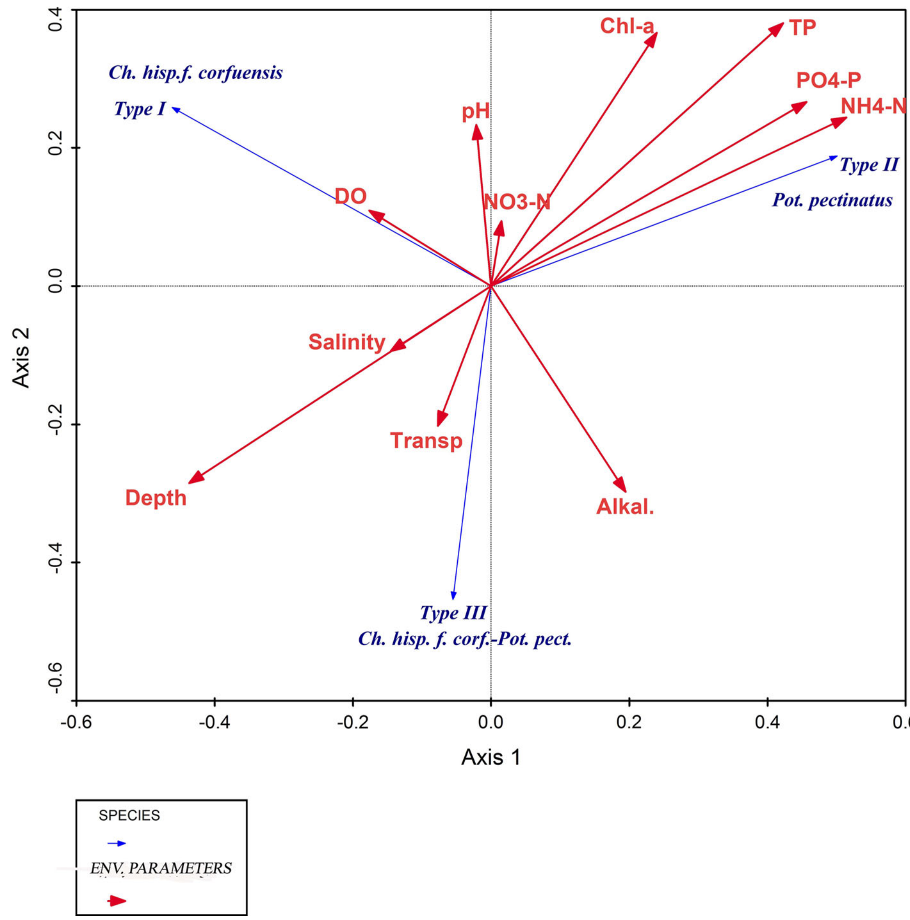

Fig. 5 The results of RDA analysis revealed the relations of submerged aquatic species of *Chara hispida f. corfuensis* and *Potamogeton pectinatus* with the environmental parameters of pH, salinity, Transparency, Depth, Alkalinity, Chl-α, NO_3-N, NH_4-N, PO_4-P, TP, DO, as well as with the three different sediment Types (I, II, III) distinguished in Kaiafas lagoon during the sampling period

Table 3 The seven distinct echo types revealed in Kaiafas lagoon by the application of the innovative side scan sonar technique and the characteristic macrophytic species observed in the lagoon's substrate after ground truthing survey

Echotypes	Depth (m)	Areal extent (% of total lagoon floor)	Ground truth sites (*n*)	Sediment Type	Species
Echotype 1	<3	8.0	2	Type I	*Potamogeton pectinatus*
Echotype 1a	<3	10.0	1	Type II	*Potamogeton pectinatus*
Echotype 2	>3	7.0	2	Type I	*Chara hispida* f. *corfuensis*
Echotype 3	>3	1.4	1	Type I	*Chara hispida* f. *corfuensis*
Echotype 4	>3	2.9	2	Type I	*Chara hispida* f. *corfuensis*
Echotype 5	>2.5	1.8	0	Type III	*Potamogeton pectinatus Chara hispida* f. *corfuensis*
Echotype 6	>2.5	27.4	3	Type I	*Chara hispida* f. *corfuensis*

water. ET1a presented similar acoustic characteristics, though this echo type exhibited higher homogeneity than that of ET1. The ground truthing sampling procedure revealed that lagoon bottom was covered at 100 % by *Potamogeton pectinatus* as also represented by the above echo types. ET1 was dominant at the southeast and central part of the lagoon and covered the 16 % of the total surveyed lagoon floor. ET1a was restricted to relatively small areas along the western and northern shoreline of the lagoon and covered about 5 % of the total insonified lagoon floor.

ET2 presented homogeneous and high reflectivity, though lower in comparison to the previous ETs. Locally regions of limited extent and of low reflectivity appeared within ET2 indicating probably vegetation cover gaps. The ground truth sampling revealed that ET2 represented areas of the lagoon floor covered by 40–50 % of *Chara hispida f. corfuensis*. This echo type was located at the southeast

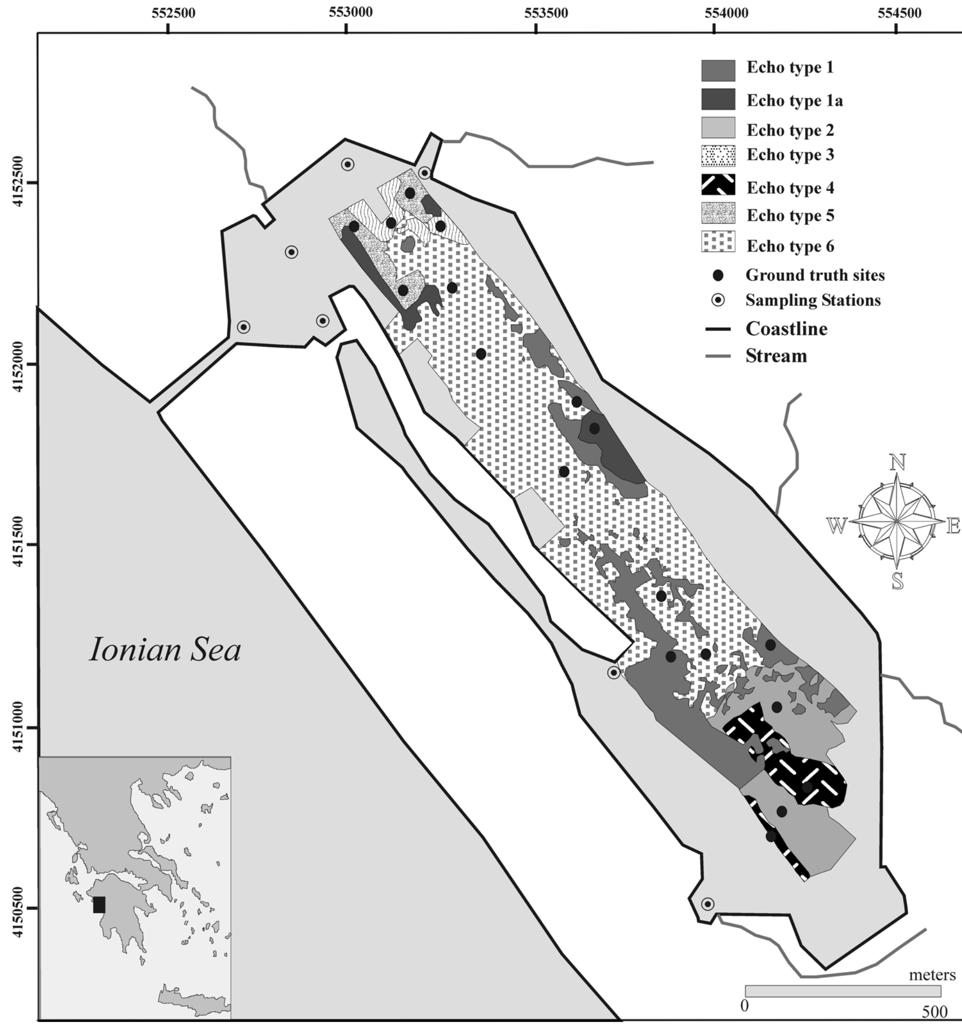

Fig. 6 The distributional pattern of the seven different characteristic echo types (ET) (a) ET1, (b) ET1a, (c) ET2, (d) ET3, (e) ET4, (f) ET5 and (g) ET6 identified in the Kaiafas lagoon floor by the implementation of the side scan sonar technique during the monitoring period

part of the lagoon and represented the 14 % of the studied lagoon floor.

The echo types ET3, 4, 5 and 6 showed a patchy pattern which consists of high reflectivity patches in a low reflectivity background. The patchy pattern was more intense on the types ET5 and 6 followed by ET3 and 4 (Figs. 6 and 7). Sampling procedure showed that ET3 and ET4 represented areas covered by *C. hispida f. corfuensis* with coverage of 80–100 % and 20–30 %, of the bottom respectively. ET3 and ET4 were restricted to relatively small areas along the northern shoreline and the southern parts of the lagoon, covering only the 2.7 and 5.7 % of the studied total area respectively.

ET5 was characterized by alterations of high and low reflectivity patches. This echo type represented areas where *C. hispida f. corfuensis* and *P. pectinatus* co-existed. ET5 was located to the northwestern part of the lagoon and covered 3.6 % of the insonified lagoon floor.

Finally, ET6 was also characterized by a patchy acoustic pattern which appeared more intense in relation to ET2–ET4 and represented *C. hispida f. corfuensis* with total coverage 100 % of the bottom (Figs. 6 and 7). It covers the 54.7 % of the lagoon floor.

Discussion

Eutrophication of coastal ecosystems resulted in water quality impairment and loss of aquatic biota (Roselli et al. 2009). However, shallow coastal environments support high densities of benthic plants and periphyton and as a result, display low phytoplankton biomass due to their competition for nutrients with the benthic producers. Thus, submerged benthic plants are playing an important role in the total primary production, as well as in the nutrient dynamics.

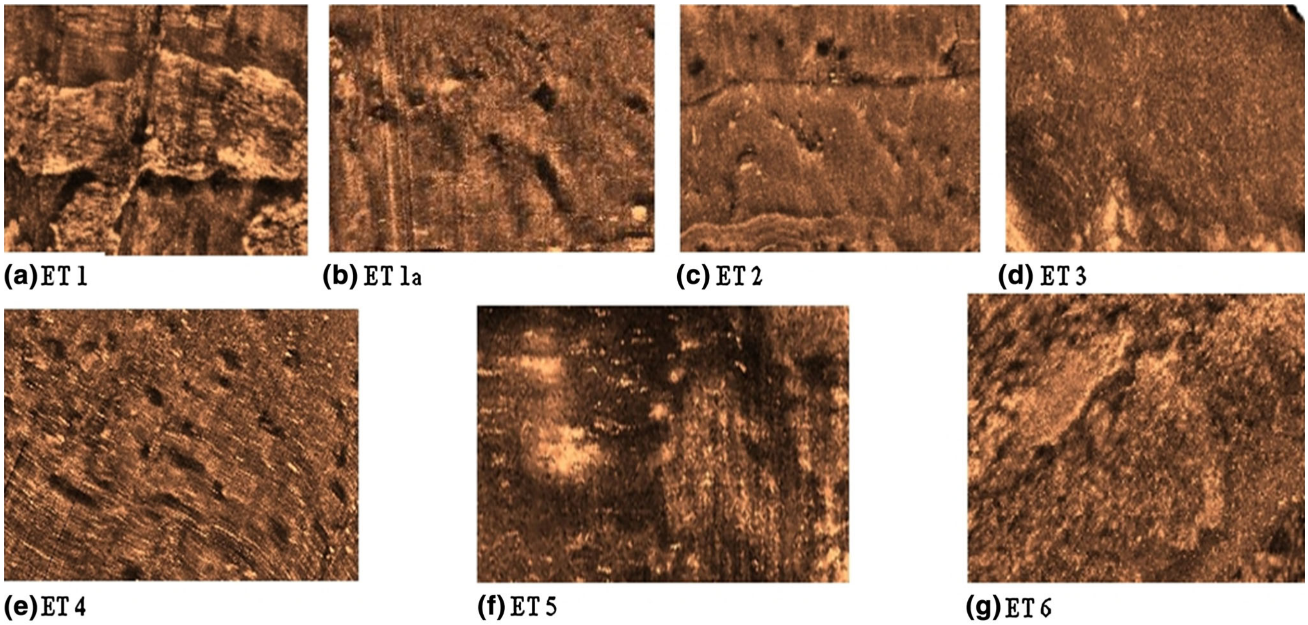

Fig. 7 Images derived after the application of hydroacoustic method (side scan sonar, SSS) applied in Kaiafas lagoon floor during the monitoring period, with the characteristic echo types (ET) **a** ET1, **b** ET1a, **c** ET2, **d** ET3, **e** ET4, **f** ET5 and **g** ET6 and macrophytic coverage

The combination of numerous forcing factors such as environmental parameters, sediment characteristics and the distribution and abundance of submerged macrophytes indicated significant space–time variability of the hydrobiology of Kaiafas lagoon. The differences in the seasonal and annual variability of the physico-chemical processes in the lagoon was evidenced by the different ranges of variation in the principal components analysis (PCA) and the differences in the correlations of nutrients with secondary and response variables.

In case of Kaiafas lagoon, the low salinity gradient (>20 ‰) affected the presence and abundance of submerged macrophytes (Christia and Papastergiadou 2007). Moreover, depth and transparency were also associated with species distribution in multivariate analysis. Depth limits of coastal macrophytes are largely regulated by transparency and also by levels of nutrient concentration and load (Greve and Krause-Jensen 2005). Sediment characteristics are likely to play a role in this regulation. Nutrient availability is partially determined by sediment types as pore water nutrient concentrations depend on grain size and sediment origin (Holmer et al. 2001). Coarse-grained carbonate sediments have larger phosphate availability due to the sediments limited adsorption capacity, compared to fine-grained sediments. Moreover, carbonate sediments are generally low in organic matter content (Koch 2001). The sandy mud substrate in Kaiafas lagoon showed maximum organic carbon content, as referred also in other Mediterranean coastal lagoons (Voutsinou-Taliadouri et al. 1987; Karageorgis et al. 2012). Elevated Corg values can be attributed to decomposition of benthic vegetation, limited water renewal and possibly high productivity.

At the northern part of the lagoon, the clear increasing trend of heavy metals concentrations with the increase of sediment core depth is probably related to the formation of diagenetic pyrite by bacterially-mediated sulphate reduction due to the reduced nature of the deeper sediments (Morse 1994). Enrichment of Cr, Cu and Pb in the upper portion of the sediment core at the southern part of the lagoon is related to sediment contamination as a consequence of the entering of the wastewater fluxes from the neighbouring town of Zacharo. The heavy metal concentration levels of Cu, Pb, Co, Ni and Cr in Kaiafas are comparable and/or slightly higher/lower to those of Greek and Mediterranean lagoons. The Zn values of the sediments in Kaiafas lagoon are always lower than those of the others lagoons (Papatheodorou et al. 2002; Rigollet et al. 2004).

Furthermore, water chemistry showed differences on seasonal and annual scale. These differences were related to alterations in nutrients of N and P, as well as of Chl-α and were probably associated with anthropogenic activities such as intense runoff of agricultural activities (in spring) and the fluxes of the wastewater treatment plant of Zacharo city.

In tradition, physicochemical parameters have been the basis for the most common indicators of trophic state in lentic ecosystems (e.g. OECD; Carlson's Trophic State Index), which were mainly developed for northern temperate lakes. In these ecosystems there is still much work to do concerning the validation of trophic state indices (Coelho et al. 2007; Salas et al. 2008) and the development and application of new tools, integrating the vulnerability of the system to the human impact. In Mediterranean aquatic ecosystems, it is still not well established which physicochemical and/or biological indicators should to be used for the assessment of the ecological status. Nonetheless, in the last decades the applicability of these indices for transitional waters has been questioned, and the eutrophication concept has evolved (Cloern 2001). Therefore, the evaluation of trophic status in coastal lagoons is very difficult as they are heterogeneous, variable, unstable and dynamic productive systems (Coelho et al. 2007; Salas et al. 2008).

In that sense Carlson's TSI index constituted a good approach for the ecological evaluation of the trophic status of Kaiafas lagoon due to its particular characteristics of high depth, low salinity and high confinement from the Ionian Sea. As regards the values of TSITP and TSISD Kaiafas lagoon is classified as hypertrophic, while the results of TSIChl-*a* indicated mainly oligotrophic conditions. High TSITP values were common in Mediterranean coastal lagoons as referred to the Ter Vell (Badosa et al. 2008) and Ricarda, Cal Tet and Ca l' Arana lagoons (Cañedo-Argüelles et al. 2012).

However, into several stressed systems (Newton et al. 2003; Coelho et al. 2007; Badosa et al. 2008; Cañedo-Argüelles et al. 2012) low Chl-*a* values were also reported, pointed out oligotrophic conditions, indicating that light availability is more likely to limit phytoplankton growth than N and P. Turbidity and light limitation that was enhanced by wind-induced sediment resuspension and prolonged flooding periods (Viaroli and Christian 2003), may cause the chlorophyll-*a* to fall below that expected from the nutrient levels.

In the current study, the relationship between TP and Chl-*a* may be closely dependent on the presence of suspended material. Solid material in suspension may reduce the production of Chl-*a* by limiting both the availability of light for photosynthesis and soluble phosphorus, the latter as a result of the adhesion of phosphate to solid particles (Ferris and Tyler 1985). The phosphorous was released as a result of the decomposition of macrophyte biomass that is promptly re-used by phytoplankton. In this environment, phosphorous is stored and subsequently released by sediments mainly in the form of phosphates and plays a significant role as a limiting factor in regulating phytoplankton assemblages in the water column above (Jordan et al. 2008).

Numerous studies showed that charophyte and pondweed could both exist at a given nutrient level and exploit nutrient resources in the sediment and the water column, however, a nutrient depletion was observed above charophyte vegetation. Fully submerged macrophytes such as charophytes, can strongly influence water transparency and are less susceptible to resuspension due to their preference to sandy substrate (van den Berg et al. 1999; Kufel and Kufel 2002). According to Koch (2001), on the other hand, when aquatic plants cover the entire water column, current velocities were efficiently reduced and suspended sediments can settle in areas where the water depth is larger than the meadow height. In that case, both resuspension and deposition can occur simultaneously and affect the light penetration, which is important for charophytes, since they use more bicarbonate at higher light intensity. On the contrary, pondweeds with canopies near the water surface hinder light penetration through shading and seems to have little positive effect on water chemistry (van den Berg et al. 1999).

The nutrient content of water in Kaiafas lagoon was lower in areas where charophytes were abundant. *Chara* primarily utilizes water column nutrients (Kufel and Kufel 2002; Hidding et al. 2010), while pondweeds are thought to rely on sediment for most of their nutrients (van den Berg et al. 1999), although not exclusively. For example, the silt and clay content (Type II) which was positively correlated with the absorption capacity of phosphorus (Specchiulli et al. 2010) showed higher values over *P. pectinatus* meadows.

The higher nutrient levels in the southern part of the lagoon (Fig. 2) and the increasing human activity in its catchment area were also indicated by the higher TSI index (Fig. 3). The stressed conditions observed especially into this part of the lagoon could potentially cause an indirect negative effect on the growth of charophyte. *Chara* colonized deeper sites in clear water, while *P. pectinatus* colonized deeper sites in turbid water. Canopy forming angiosperms like *P. pectinatus* are known for their tolerance to high turbidity and therefore, are even able to survive in hypertrophic conditions (van Donk and van der Bund 2002). The conditions inside macrophyte beds may increase denitrification, contributing to a decreased availability of nitrogen for phytoplankton growth (van Donk and van der Bund 2002); thus water within *Chara* meadows contained significantly less Chl-α and nutrient concentrations. However, the development of *Potamogeton* in the Kaiafas lagoon would further resulted in a decline of the intensity of light reaching the substrate/bottom. This fact has been reported by several authors mainly in western Mediterranean lagoons where there are many published papers, who have pointed out the replacement of *Chara* sp. by species of angiosperms and especially pondweeds, whose development is encouraged by nutrient enrichment of the waters (van den Berg et al. 1999; Kufel and Kufel 2002).

In Kaiafas lagoon almost 100 % of the area acquired with the SSS is located in depth ranging from 2.5 to 3.3 m.

Shallow water depth in Kaiafas lagoon was considered as limiting factor for the application of this technique due to the contamination of the returned SSS signal by multipath returns and the demanding procedure which requires the towfish to be towed short distance behind the vessel and very close to the floor. However, the short length of the towing cable permitted more accurate positioning of the SSS imagery and thus more precise mapping of the submerged aquatic vegetation limits (Manley and Singer 2008). As far as we know, this hydroacoustic survey is the first application of acoustic classification approach in such shallow waters with the work of Preston et al. (2000) being an exception.

In Kaiafas lagoon, the gathered sonar data in conjunction with targeted ground truthing sampling revealed the distributional pattern of *Chara hispida f. corfuensis* and *Potamogeton pectinatus* at the lagoon floor. *C. hispida f. corfuensis* appeared to be the dominant species, which was presented by homogeneous or patchy acoustic patterns on sonographs and recorded at sandy substrate (Type I). However, the echo types represented by *P. pectinatus* (Fig. 7) were characterized by sharp limits with higher elevation compared to the neighboring lagoon floor and to more silt and clay substrate (Type II). This is in accordance with the ability of *P. pectinatus* to form a canopy of leaves near the water surface. Furthermore, *P. pectinatus* was found at the limits of the lagoon and at the southern part of the lagoon showing the tolerance of this species under stress environmental condition and anthropogenic impacts.

The application of SSS has shown up its advantages in Kaiafas lagoon for distinguishing *P. pectinatus* and *C. hispida f. corfuensis* in terms of extent and composition regarding the bottom relief in a shallow (<3.5 m) lagoon environment. This method has been recently questioned by several authors for its efficiency for the discrimination of seabed cover (van Rein et al. 2011) as the SSS data were usually analyzed by image interpretation techniques (often, visual interpretation), so results can be subjective (Anderson et al. 2008). In the shallow Pappas lagoon (W. Greece) SSS showed that the patchy acoustic character was associated with the vegetation coverage of the floor and was influenced not only by the grain-size characteristics of the sediments but also by the biotic characteristics of the lagoon-bed (Papatheodorou et al. 2012). However, the ability of acoustic classification system showed its advantages in discriminating different sediment types in a relatively monotonous fine-grained floor of a lagoon where the superficial sediment gradually changes from fine sand to mud with silt and clay content.

The findings of the current study were based on results obtained by data integration which can be an effective key analytical tool to improve the knowledge of coastal complex environments and address conservation strategies. Gathering a large quantity of different data, with diverse techniques in the same framework could significantly improve the interpretation of the overall environmental characteristics. The enhanced details in discrimination, areal coverage and mapping of benthic macrophytes vegetation by the use of SSS technology combined with targeted ground truthing sampling, represent a substantial improvement over the previous mapping approaches generated from sampling on a grid or interpretations of aerial photographs. The SSS technology showed a high applicability in turbid and shallow environments, such as a lagoon, and can be considered as a cost effective approach since it can be done at both high speed and over a broad path width using small boat (Gilvear et al. 2004).

Therefore, as the lagoon is influenced by the freshwater inputs and also suffers from wastewaters inflows from the catchment's area further studies should also include the changes of depth distribution limit of macrophytes which constitutes a valuable indicator of human impacts, and the subsequent effect of eutrophication in the loss of taxa and biotic elements. The integration of acoustic, physico-chemical and sediment data together with advanced data processing systems leads to a better understanding of the biological processes and metabolism in a shallow lagoon in different spatial and temporal scales and will contribute to future sustainable conversation efforts that minimize human influences in the lagoon.

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