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Analysis of Phytoplankton Community Structure Using Similarity Indices: A New Methodology for Discriminating Among Eutrophication Levels in Coastal Marine Ecosystems

G. ARHONDITSIS*,†
M. KARYDIS
G. TSIRTSIS
Department of Marine Sciences
University of the Aegean
Sapfous 5, 81100 Mytilene, Greece

ABSTRACT / Nine similarity indices based on phytoplankton community structure were examined for their sensitivity to assess different levels of eutrophication. Two phytoplankton data sets, one from an open coastal system and one from a semi-enclosed gulf, associated with different nutrient dynamics and circulation patterns were used for evaluating the indices. The results have shown that similarity indices, measuring interspecific association and resemblance of phytoplankton communities between enriched areas and control sites, were effective for detecting spatial and temporal dissimilarities in coastal marine ecosystems. The structure of the oligotrophic habitat as a potential source of ambiguity for the results was discussed, whereas the validity ranges and the potential applicability of this method were deemed to be dependent on the size of the fraction of the common species among the samples, and the similarity of the classification patterns resulted from this subcategory and those extracted from the overall community data. Furthermore, the study provides a new technique based on the use of the "Box and Whisker Plot" designed to distinguish opportunistic and rare phytoplanktonic species. The similarity indices, applied solely to the dominant species abundance, were more sensitive to resolve eutrophic, mesotrophic and oligotrophic conditions. This procedure can be proposed as an effective methodology for water characterization and can also be used as a qualitative tracer for detecting renewal processes of coastal marine ecosystems.

One of the complex environmental issues often occurring on coastal ecosystems is the assessment of eutrophication levels, a problem that is further complicated by seasonal trends and the difficulty in distinguishing between natural and human-induced stresses in the environment (Lipiatou and Cornaert 1999). Various attempts to develop more sensitive tools for pollution assessment and coastal management have focused on the structural changes of communities, which is the most reliable level of biological organization for environmental impact studies (Clarke 1993, Warwick 1993). The term "community" is rather general and may include various types of aquatic communities such as phytoplankton, periphyton, macrophyte, zooplankton, benthic invertebrates and fish communities. Although the effects of marine eutrophication expand to all the above types of community, depending on the phase of cutrophic conditions, the primary stages concern mainly the phytoplankton (Gray 1992). Therefore, if new methodologies are going to be used as "early" warning systems to detect eutrophic trends in the marine environment, they should focus on phytoplankton community changes during the phase of the initial effects (Karydis and Tsirtsis 1996).

The evaluation and systemization of phytoplankton community structural analyses have been supported extensively by ecological indices. Numerous diversity, evenness and dominance indices have been reviewed with special relevance to aquatic ecosystems and coastal marine eutrophication (Vollenweider and others 1992, Spellerberg 1993, Death and Winterbourn 1995, Karydis and Tsirtsis 1996, Tsirtsis and Karydis 1998). However, it has been found that most of the ecological indices are not producing consistent results when applied to different phytoplankton data-sets and they are also lacking sensitivity at the intermediate -mesotrophic levels of environmental stress (Karydis and Tsirtsis 1996). These indices are

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†Present address: Department of Civil and Environmental Engineering, University of Washington, 313B More Hall, Box 352700, Seattle, Washington 98195, USA.

*Author to whom correspondence should be addressed; *email:* georgea@u.washington.edu

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not sufficient in describing either the initial structure of the community or its response to external stress factors, properties that are essential when changes in phytoplankton communities are used for water quality assessment (Boyle and others 1990).

Consequently, the development of analytical procedures for the study of phytoplankton community structure should take into account both the significance of the baseline information collected from unimpacted (control) sites and the divergence between these reference levels and data from affected areas. The application of the similarity indices seems to be more consistent in this context. They can be used to measure the compositional overlap along with the species' abundance differences among samples from enriched and control areas, which is likely to lead to a sensitive methodology that detects eutrophic trends and properly classifies different pollution levels.

The present study basically aims at evaluating the effectiveness of nine similarity indices, measuring interspecific association and resemblance of phytoplankton communities between polluted and control sites, as estimators of different levels of marine quality. The selection of the reference area was proven to be critical and therefore an objectivity criterion is proposed in order to secure validity of the results. The sensitivity of these similarity indices was tested against the whole data-set and solely to the subset of the dominant species that promptly respond to nutrient enrichment. The discrimination between dominant and rare phytoplankton species is the result of a technique focused on the detection of outlying values. The basic purpose for the development of the present methodology was to establish an effective classification scheme with high resolution under mesotrophic conditions, when there are still wide margins for alternative management practices in the coastal zone.

Methodology

Study Areas

Gulf of Gera. The gulf of Gera, Island of Lesvos, Greece, is a semi-enclosed water body with a mean depth of 10 m and a total volume of 0.9×10^9 m^3 (Figure 1). The surrounding 194.01 km^2 drainage area, mainly cultivated with olive trees and inhabited by 7064 people according to the 1991 census, influences this shallow marine environment. Nutrient fluxes from agricultural run-off and non-point sources, especially during the winter period, vary from 40 to 60% of the total nutrient stock (Arhonditsis and others 2000). Table 1 shows estimates of nutrient exports from the surrounding watershed due to soil erosion and surface runoff (Arhonditsis and others 2002). The most important point discharges in the water body are untreated domestic effluents and wastewater from local industrial activities mainly a by-product from the processing of oil crops by 'centrifugal' type oil presses. Data collection was based on 17 cruises carried out on a monthly basis from June 1996 to October 1997. Samples were collected from eight sampling stations [GG1] to [GG8] shown in Figure 1A. Six stations [GG3]-[GG8] were located inside the gulf and the other two were situated in the entrance (station [GG2]) and outside of the gulf (station [GG1]), in order to monitor transport processes and reference levels, respectively. Phytoplankton samples were fixed with Lugol Iodine and the phytoplankton enumeration and classification were carried out on an inverted microscope (Lund and others 1958). Analytical procedures used for the determination of the physical (salinity, temperature), chemical (nitrate, nitrite, ammonium, dissolved organic nitrogen, phosphate, silicate) and biological (chlorophyll a and total bacterial number) variables have been described elsewhere (Arhonditsis and others 2000).

Coastal area of the city of Rhodes. The second data collection was carried out on a monthly basis from 1 m depth during May 1983-April 1984 at 10 stations (Figure 1B) situated along the coastal area of the city of Rhodes, Island of Rhodes, Greece, as described in previous papers (Karydis and Coccosis 1990, Karydis 1992, Karydis 1994). Stations [RH3], [RH4] and [RH5] were located in the vicinity of harbors, defining the upper eutrophication limits in the area. Two stations ([RH7] and [RH9]) located about 1 mile offshore (in an area with intense circulation and depth greater than 200 m) were chosen as the control sites (the baseline information of the system); the remaining stations were spaced out near shore used for swimming and other recreational activities. Sampling network, analytical procedures and results of the measured variables (temperature, salinity, oxygen, nitrate, nitrite, ammonium, phosphate and chl *a*), have been described in detail elsewhere (Karydis and Coccosis 1990).

The eligibility of the two areas and the specific networks used for the present eutrophication study were tested by isolating the effects of the physical environment and verifying the dominant role of the external loading to the observed patterns of the water quality variables. For this purpose, analysis of covariance (ANCOVA) and *t* test (Zar 1999) were used to compare the variability attributed to the physical parameters with the

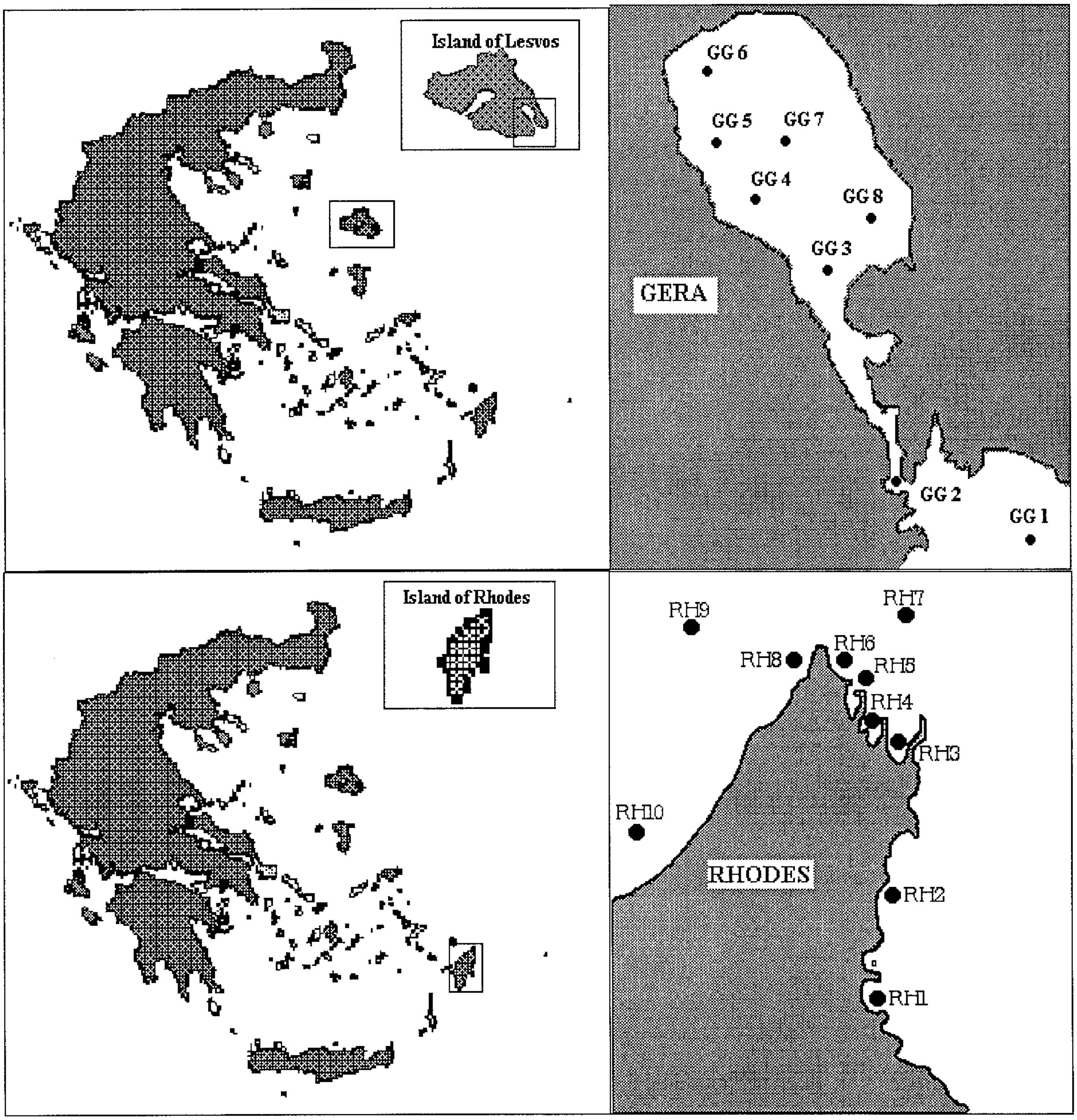

Figure 1. (**a**) The gulf of Gera, Island of Lesvos, Greece: station locations. (**b**) The coastal area of the city of Rhodes, Island of Rhodes, Greece: station locations.

residual variability (mostly due to nutrient discharges) and quantify their contribution to the total spatial heterogeneity of the eutrophication parameters (nutrients, chlorophyll *a*).

Phytoplankton Community Similarity Indices

Nine similarity indices, quantifying the contribution of each species to the total phytoplankton biomass, were used as estimators of the resemblance among discharged areas and control sites. The following similarity indices were used (Whittaker 1973, Legendre and Legendre 1983, Valiela 1995):

(1) Gleason's index: $$SI_{GL} = \frac{\sum_{i\epsilon T} (x_i + y_i)}{\sum_{i} (x_i + y_i)}$$

Table 1. Cumulative monthly exports (kg) of dissolved-phase nitrate, ammonium, and phosphate and solid-phase ammonium, and phosphate from Gera's watershed*

	Dissolved phase			Solid phase	
Date	Nitrate	Ammonium	Phosphate	Ammonium	Phosphate
Nov-95	45	23	19	8.7	5.8
Dec-95	130	84	121	13.5	10.2
Jan-96	388	172	122	45	32
Feb-96	9574	4509	3295	434	176
Mar-96	165	88	55	88	33
Apr-96	955	418	272	167	172
Sep-96	0	0	0	68	39
Oct-96	0	0	0	23	11
Nov-96	1745	552	825	444	201
Dec-96	5988	2576	2119	223	155
Jan-97	544	192	202	134	78
Feb-97	420	209	103	78	49
Mar-97	311	119	89	58	32
Apr-97	95	55	45	44	45
Oct-97	72	49	36	99	36
Nov-97	1296	502	392	185	123
Dec-97	5630	1582	905	345	75
Jan-98	590	337	420	134	76
Feb-98	420	202	203	78	30
Mar-98	162	55	69	55	45
Apr-98	99	54	40	34	33

*Study period was September 1995-May 1998 and the nutrient loading during the non-reported months was negligible. For further details, see Arhonditsis and others 2002.

(2) Kulczynski's index:

$$SI_{KU}=\frac{1}{2}\left(\frac{1}{\sum_i x_i}+\frac{1}{\sum_i y_i}\right)\cdot\sum_i \min(x_i, y_i)$$

(3) Ruzicka's index:

$$SI_{RU}=\frac{\sum_i \min(x_i, y_i)}{\sum_i x_i+\sum_i y_i-\sum_i \min(x_i, y_i)}$$

(4) Pandeya's index: $SI_{PA}=\dfrac{\sum_{i\epsilon T}(x_i+y_i)}{\sum_i (x_i+y_i)+\sum_{i\epsilon T}|x_i-y_i|}$

(5) Ellenberg's index:

$$SI_{EL}=\frac{\sum_{i\epsilon T}(x_i+y_i)}{2\cdot\sum_{i\epsilon U}x_i+2\cdot\sum_{i\epsilon V}y_i+\sum_{i\epsilon T}(x_i+y_i)}$$

(6) Whittaker's index of association:

$$SI_{WH}=\frac{1}{2}\sum_i\left|\frac{x_i}{\sum_i x_i}-\frac{y_i}{\sum_i y_i}\right|$$

(7) Canberrra metric: $SI_{CA}=\sum_i\left(\dfrac{|x_i-y_i|}{(x_i+y_i)}\right)$

(8) Morisita's index: $SI_{MO}=\dfrac{2\cdot\sum_{i\epsilon T}x_i\cdot y_i}{(D_1+D_2)\cdot\sum_i x_i\cdot\sum_i y_i}$

(9) Bray-Curtis' index: $SI_{BC}=\dfrac{\sum_i|x_i-y_i|}{\sum_i(x_i+y_i)}$

where x_i, y_i are the abundances of the ith phytoplankton species in the two sampling sites, T is the subset of species occurring in both samples, U and V are those occurring in one or other only and D_1, D_2 are the Simpson's index of the two samples, which is a probabilistic measure expressing the likelihood that two individuals sampled randomly and independently from a community will be found to belong to the same species. The Simpson's equation is $D=\dfrac{\sum_i n_i\times(n_i-1)}{n\times(n-1)}$ and n_i, n are the number of the ith species and the total number of individuals in the sample, respectively.

Data Manipulation and Classification

Gulf of Gera. The data set consisted of 232 sampling units, taken from standard depths of the eight stations during a time period of 17 months. The total number of phytoplanktonic species present was 139. The similarity indices were used to compare the monthly subsets (containing samples from the standard depths) of each of the internal stations [GG2]-[GG8] with the respective depths of the reference site [GG1]. Thus, a monthly value was assigned to the seven stations of the gulf reflecting the mean deviations of their phytoplankton communities from the baseline conditions of the study area (9 indices × 7 stations × 17 months).

Table 2. Summary statistics of temperature, salinity[a], nutrients, chl a concentrations and species number (ranges and mean values) recorded at the sampling sites of the Gulf of Gera (stations GG1–GG8) and the coastal area of the city of Rhodes (RH1–RH10)

Sites	Stations	Temperature (°C)	Salinity (‰)	Nitrate (μM)	Nitrite (μM)	Ammonia (μM)	Phosphate (μM)	Org. nitrogen (μM)	chl α (μg/L)	Species number
Gulf of Gera	GG1	(12.37–21.59)	(38.99–39.15)	(0.06–0.76)	(0.01–0.26)	(0.07–0.79)	(0.05–0.29)	(1.01–23.54)	(0.02–0.73)	(6–32)
		18.47	39.04	0.26	0.06	0.24	0.13	7.83	0.18	14
	GG2	(12.16–24.14)	(38.22–39.49)	(0.12–1.17)	(0.02–0.17)	(0.07–2.55)	(0.06–0.27)	(0.58–14.33)	(0.03–2.00)	(8–37)
		18.68	38.93	0.42	0.08	0.43	0.14	7.96	0.58	17
	GG3	(11.00–23.99)	(38.09–40.11)	(0.09–2.01)	(0.01–0.26)	(0.11–4.94)	(0.06–0.62)	(0.98–27.99)	(0.09–2.37)	(13–24)
		18.86	38.97	0.58	0.08	1.10	0.19	8.68	0.86	19
	GG4	(10.03–25.95)	(38.09–40.19)	(0.13–2.57)	(0.01–0.34)	(0.12–5.39)	(0.02–0.41)	(0.55–16.01)	(0.15–2.05)	(11–35)
		18.69	39.12	0.65	0.10	0.88	0.18	6.26	0.85	18
	GG5	(10.11–26.13)	(37.71–40.28)	(0.12–2.45)	(0.02–0.24)	(0.12–4.20)	(0.08–0.85)	(0.17–17.88)	(0.17–2.58)	(8–25)
		18.77	39.12	0.84	0.10	0.67	0.25	7.05	1.03	17
	GG6	(10.01–26.14)	(38.01–40.23)	(0.10–2.02)	(0.02–0.56)	(0.12–4.02)	(0.08–0.59)	(4.21–18.65)	(0.04–3.03)	(8–26)
		18.90	39.12	0.71	0.13	0.76	0.21	8.94	1.19	17
	GG7	(10.58–24.78)	(38.17–40.25)	(0.09–1.48)	(0.01–0.17)	(0.05–6.62)	(0.07–0.51)	(1.70–20.51)	(0.06–3.07)	(9–22)
		18.53	39.17	0.53	0.07	0.89	0.19	8.44	1.02	16
	GG8	(9.88–25.84)	(38.17–40.22)	(0.12–1.09)	(0.02–0.44)	(0.06–3.01)	(0.07–0.35)	(1.20–23.45)	(0.03–2.13)	(11–31)
		18.70	39.10	0.52	0.09	0.64	0.15	8.09	0.89	17
Rhodes	RH1	(16.10–25.80)		(0.16–1.46)	(0.00–0.10)	(0.21–2.01)	(0.00–0.30)		(0.02–0.19)	(9–23)
		20.60		0.68	0.05	0.82	0.09		0.10	18
	RH2	(16.50–25.80)		(0.16–0.99)	(0.01–0.11)	(0.14–1.64)	(0.01–0.13)		(0.01–0.15)	(12–24)
		20.57		0.45	0.05	0.58	0.06		0.11	16
	RH3	(16.80–25.80)		(0.43–5.37)	(0.02–0.14)	(0.33–0.83)	(0.01–0.10)		(0.07–0.33)	(14–28)
		20.27		2.51	0.09	0.52	0.06		0.18	19
	RH4	(16.70–26.50)		(2.12–22.79)	(0.04–0.39)	(0.21–1.22)	(0.04–0.18)		(0.05–1.18)	(14–28)
		20.62		6.25	0.13	0.59	0.07		0.43	18
	RH5	(16.20–26.50)		(1.01–4.89)	(0.06–0.16)	(0.31–0.90)	(0.03–0.13)		(0.21–1.48)	(14–28)
		20.68		3.00	0.10	0.61	0.07		0.65	19
	RH6	(16.60–26.50)		(0.17–1.23)	(0.00–0.10)	(0.23–6.99)	(0.01–0.23)		(0.08–0.47)	(7–30)
		20.88		0.60	0.05	1.06	0.07		0.18	17
	RH7	(16.60–26.50)		(0.01–0.79)	(0.00–0.14)	(0.04–0.92)	(0.01–0.14)		(0.01–0.17)	(9–21)
		21.01		0.35	0.05	0.44	0.06		0.08	14
	RH8	(16.80–26.50)		(0.09–0.92)	(0.00–0.11)	(0.19–2.74)	(0.02–0.20)		(0.01–0.15)	(8–26)
		20.88		0.42	0.04	0.62	0.07		0.08	18
	RH9	(16.80–26.50)		(0.04–0.51)	(0.00–0.11)	(0.15–1.44)	(0.01–0.13)		(0.01–0.17)	(5–20)
		21.10		0.28	0.04	0.59	0.06		0.08	13
	RH10	(16.50–26.50)		(0.01–2.76)	(0.00–0.10)	(0.08–1.75)	(0.01–0.11)		(0.03–0.13)	(9–20)
		20.72		0.51	0.04	0.50	0.05			13

[a] Salinity in the coastal area of the city of Rhodes was measured on a seasonal basis (n = 5) with a range of 38.90–39.39$\%_0$ and mean value of 39.13$\%_0$.

Coastal area of Rhodes. In a similar way, the calculation of the similarity indices was based on a data set that consisted of 120 sampling units (10 stations × 12 months) and the total number of phytoplanktonic species was 197. The application of these indices has led to a final data matrix of 9 columns (indices) and 96 rows (8 × 12 sampling units), measuring the mean resemblance per month of the two control stations [RH7] and [RH9] with each of the other eight sampling sites.

Cluster analysis was applied for each index to study the grouping of the stations. The Euclidean distance was used as a similarity measure and the group average distance was chosen as the clustering algorithm since this clustering technique introduces relatively little distortion to the relationships between stations. Finally, Analysis of Similarities (ANOSIM) was used as a conservative test to determine if stations that appear to be grouped together in separate clusters of the dendrograms form statistically significant distinct groups (Clarke and Green 1988).

Results

Whole Body of Data

The mean annual levels of temperature, salinity, nitrate, nitrite, ammonium, phosphate, organic nitrogen and chlorophyll *a* concentrations in the eight stations of the gulf of Gera are presented in Table 2. It can be observed that the six internal stations [GG3]-[GG8] showed higher nutrient and chlorophyll levels compared with the control site [GG1]; these mean values are characteristic of a marine environment that can be classified as mesotrophic with eutrophic trends according to a proposed scaling for the Aegean Sea (Ignatiades and others 1992). The respective concentrations at

Table 3. Analysis of covariance (ANCOVA) and least square difference test for detecting differences among the sampling sites of the Gulf of Gera for total inorganic nitrogen (TIN), phosphate and chlorophyll *a* concentrations

Sources of variance	Degrees of freedom[a]	F ratio	Level of significance	Overall adjusted r^2	Groups
TIN					
Stations	7/119	3.87	0.00[b]	0.72	*A*: GG1 *B*: GG2
Temperature	1/119	0.70	0.40		*C*: GG3-GG4-GG5-GG6-GG7-GG8
Salinity	1/119	0.34	0.56		
Phosphate					
Stations	7/119	6.73	0.00[b]	0.89	*A*: GG1 *B*: GG2-GG3
Temperature	1/119	4.38	0.01[b]		*C*: GG4-GG5-GG6-GG7-GG8
Salinity	1/119	2.63	0.10		
chla					
Stations	7/119	6.23	0.00[b]	0.73	*A*: GG1 *B*: GG2
Temperature	1/119	5.14	0.03[b]		*C*: GG3-GG4-GG5-GG6-GG7-GG8
Salinity	1/119	4.82	0.08		

[a] DF of groups/DF of error.

[b] Significant value at 0.05.

station [GG2] located in the entrance of the gulf were at intermediate levels. The grouping of the stations in terms of their total dissolved inorganic nitrogen, phosphate and chlorophyll concentrations after eliminating the effects of the physical variability, was tested using the analysis of covariance where the sites' IDs were the values of the categorical variable and the physical variables (temperature, salinity) were the covariates. The sources of differences among the stations were sought by the least square difference test (LSD). The results of these statistical analyses are presented in Table 3. The physical environment (especially temperature) has a significant contribution in some cases, however, the residual variability (spatial heterogeneity) is still significant in the observed patterns of nutrients and chlorophyll (higher and statistically significant F values). The application of the LSD test has resulted in a grouping of the stations similar to the one previously described. The compositional overlap of phytoplankton species between the stations of the gulf and the control site was also remarkable, exceeding in proportion the level of 65%.

The numerical classification of the stations based on the application of the Morisita's index (randomly selected) is represented in Figure 2A. It can be seen that the internal stations of Gera formed two distinct groups: the group of stations [GG5], [GG6], [GG7] located at the northwestern part of the gulf and the group of stations [GG3], [GG4], [GG8] characterizing the southeastern spatial compartment of the area, whereas the station [GG2] at the entrance of the gulf had a discrete performance of its phytoplankton community. Similar classification patterns have resulted from the remaining similarity indices, and the statistical testing of this grouping, based on the use of the non-parametric permutation test ANOSIM, has led to its statistical confirmation at the level of 5% (Table 5). Conclusively, the similarity indices, as measures of the deviations among affected and control sites, seem to be a sensitive methodology for water characterization, enabling the detection of spatial trends in the structure of phytoplankton communities.

The mean annual values of the nitrate, nitrite, ammonium, phosphate and chlorophyll *a* concentrations in the 10 stations of the coastal area of Rhodes are also presented in Table 2. It can be seen that the stations in the harbor ([RH3], [RH4] and [RH5]) were characterized by higher nutrient and chlorophyll values. Meanwhile, the two offshore stations ([RH7] and [RH9]) exhibited the lowest concentrations, especially for nitrate and ammonium. The respective values in the remaining stations (used for swimming and other recreational activities) were at intermediate levels. The effects of the hydrodynamics and the physical variability were initially evaluated using the analysis of covariance (not presented in this paper), but the resulting r^2s were low (<30%) and therefore not sufficient for addressing the specific question. We followed an indirect approach by dividing the dataset into two subsets; the summer period (May-October) characterized by strong stratification of the waters in the area and the winter period (November-April) when the mixing is particularly strong, and comparing the grouping of the stations under the two different hydrodynamic regimes. Total

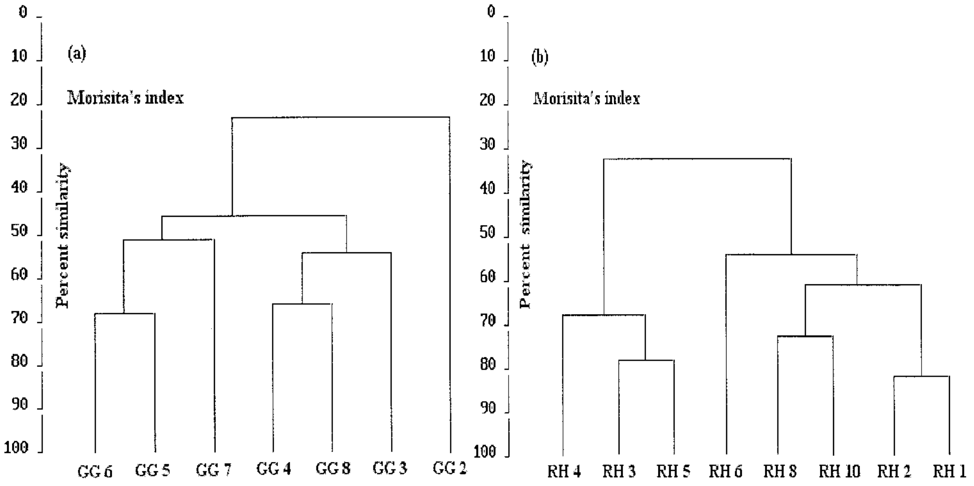

Figure 2. Grouping of (**a**) the nearshore stations of the coastal area of the city of Rhodes and (**b**) the internal stations of the gulf of Gera, based on the whole body of data. The resemblance measure among the stations and the reference sites was Morisita's index.

Table 4. Testing the difference (paired *t*-test) between stations (Rhodes) of the seasonal mean of total inorganic nitrogen*

	RH1	RH2	RH3	RH4	RH5	RH6	RH7	RH8	RH9	RH10
RH1		1.47	1.11	5.97^{b}	2.43	1.41	1.69	0.89	1.95	1.20
RH2	2.43		2.63^{a}	4.70^{b}	5.02^{b}	0.13	0.93	0.03	0.20	0.43
RH3	1.16	1.19		3.83	1.63	3.49^{a}	3.60^{a}	2.39	3.13^{a}	2.94
RH4	2.38	2.41	0.63		1.84	5.20^{b}	5.16^{b}	4.66^{b}	6.51^{b}	4.85^{b}
RH5	4.18^{b}	4.31^{b}	0.96	1.61		5.15^{b}	5.39^{b}	2.62^{a}	3.68^{a}	4.47^{b}
RH6	0.89	1.18	1.09	2.90^{a}	1.80		1.97	0.02	0.13	0.62
RH7	2.93^{a}	1.20	1.21	2.60^{a}	5.20^{b}	1.59		0.33	0.52	0.06
RH8	1.22	1.37	1.19	2.61^{a}	5.22^{b}	1.39	2.11		0.09	0.30
RH9	2.00	0.81	1.21	2.53	5.44^{b}	1.37	0.25	1.06		0.29
RH10	0.61	0.27	1.18	2.73^{a}	4.93^{b}	1.63	1.36	0.39	0.91	

*Upper triangle: comparisons between stations during the summer period; lower triangle: comparisons between stations during the winter period. Critical values for statistical significance: ^{a}(5%) = 2.571; ^{b}(1%) = 4.032; degrees of freedom = 5. Table modified from Karydis and Coccosis (1990).

inorganic nitrogen (nitrate, nitrite and ammonium) was considered as the main eutrophication parameter (Karydis and Coccosis 1990), and the seasonal means of the stations were compared using the *t*-test. Table 4 shows that during the summer period (upper triangle) significant differences were detected between the highly eutrophic stations RH4, RH5 and most of the remaining stations. Similar patterns were also observed during the winter period (lower triangle), indicating dominance of the nutrient loading over the circulation processes and the physical variability in the study area. The phytoplankton communities of the offshore and inshore stations showed a significant overlap of their species composition (>75%) and the differences in the abundance of these common species between the two sites (enriched and offshore) is one of the main factors used in the present methodology.

The application of the similarity indices in the specific area, which in fact means the comparison of the discharged stations in terms of their resemblances to the control sites, is illustrated in Figure 2B. This representation involves the classification results derived from Morisita's index, but analogous inferences were extracted from the rest of the similarity measures. It is clear that we have a clear bipolar pattern: stations [RH3], [RH4], [RH5] were clustered closely together and the remaining stations [RH1], [RH2], [RH6], [RH8] and [RH10] were clumped into a single group at a mean level varying from 45 to 55% of similarity. Table 5 presents the results of the ANOSIM test, where

Table 5. ANOSIM randomization test to confirm statistically significant differences between groups of stations

Pairwise comparisons between groups of stations	Significance (%)	Pairwise comparisons between groups of stations	Significance (%)
Gleason's index		Whittaker's index of association	
Gera: [GG3, GG4, GG8] vs [GG2]	4.76*	Gera: [GG3, GG4, GG8] vs [GG2]	4.02*
[GG5, GG6, GG7] vs [GG3, GG4, GG8]	4.12*	[GG5, GG6, GG7] vs [GG3, GG4, GG8]	3.52*
[GG5, GG6, GG7] vs [GG2]	3.98*	[GG5, GG6, GG7] vs [GG2]	2.44*
Rhodes: [RH3, RH4, RH5] vs [RH1, RH2, RH6, RH8, RH10]	3.39*	Rhodes: [RH3, RH4, RH5] vs [RH1, RH2, RH6, RH8, RH10]	2.67*
Kulczynski's index		Canberra metric	
Gera: [GG3, GG4, GG8] vs [GG2]	4.76*	Gera: [GG3, GG4, GG8] vs [GG2]	3.99*
[GG5, GG6, GG7] vs [GG3, GG4, GG8]	4.25*	[GG5, GG6, GG7] vs [GG3, GG4, GG8]	2.33*
[GG5, GG6, GG7] vs [GG2]	3.69*	[GG5, GG6, GG7] vs [GG2]	1.78*
Rhodes: [RH3,RH4,RH5] vs [RH1,RH2,RH6,RH8,RH10]	4.42*	Rhodes: [RH3,RH4,RH5] vs [RH1,RH2,RH6,RH8,RH10]	2.79*
Ruzicka's index		Morisita's index	
Gera: [GG3, GG4, GG8] vs [GG2]	4.49*	Gera: [GG3, GG4, GG8] vs [GG2]	3.46*
[GG5, GG6, GG7] vs [GG3, GG4, GG8]	4.02*	[GG5, GG6, GG7] vs [GG3, GG4, GG8]	2.79*
[GG5, GG6, GG7] vs [GG2]	3.59*	[GG5, GG6, GG7] vs [GG2]	1.31*
Rhodes: [RH3,RH4, RH5] vs [RH1,RH2,RH6,RH8,RH10]	3.67*	Rhodes: [RH3,RH4,RH5] vs [RH1,RH2,RH6,RH8,RH10]	2.57*
Pandeya's index		Bray-Curtis' index	
Gera: [GG3, GG4, GG8] vs [GG2]	4.89*	Gera: [GG3, GG4, GG8] vs [GG2]	3.94*
[GG5, GG6, GG7] vs [GG3, GG4, GG8]	4.17*	[GG5, GG6, GG7] vs [GG3, GG4, GG8]	2.56*
[GG5, GG6, GG7] vs [GG2]	3.59*	[GG5, GG6, GG7] vs [GG2]	1.68*
Rhodes: [RH3,RH4,RH5] vs [RH1,RH2,RH6,RH8,RH10]	4.16*	Rhodes: [RH3,RH4,RH5] vs [RH1,RH2,RH6,RH8,RH10]	2.92*
Ellenberg's index			
Gera: [GG3, GG4, GG8] vs [GG2]	3.50*		
[GG5, GG6, GG7] vs [GG3, GG4, GG8]	2.87*		
[GG5, GG6, GG7] vs [GG2]	1.99*		
Rhodes: [RH3,RH4,RH5] vs [RH1,RH2,RH6,RH8,RH10]	3.46*		

*Indicates significant differences between the 1% and 5% levels.

The test was applied to the results of the clustering based on the application of the similarity indices to the whole body of data.

it can be seen that all the similarity indices have resulted in the same grouping of stations and the existence of these spatial discontinuities was confirmed statistically at the level of 5%.

However, the intra- and interspecific differences in abundance levels among discharged and control sites are not always associated with eutrophication phenomena. The dynamics of the phytoplankton populations are also influenced by several ecological factors of varying importance with respect to the spatial scale of interest, such as the requirement for a specific vitamin or a trace element, the demand for a certain substrate if the species forms a sedentary stage, the annual variability of salinity, and temperature and current transport (Pagou and Ignatiades 1988). The basic concept of the present method presupposes that all these factors, virtually a kind of environmental "noise" for this study, are isolated and that the sampling points used for spatial control objectively provide the baseline information for the communities encountered in the coastal areas. Thus, the selection of the unaffected stations seems to be a potential source of ambiguity for interpretation of the results and a key issue for the reliability of this method.

The most rational practice to define the structure of the oligotrophic habitat is to seek sampling sites located in the vicinity of the coastal system. However, in cases where the distinction between environments with different trophic potential and different physical structure is not clear, it seems likely that most of the abovementioned factors are partly removed from the study by utilizing the overlapping fraction of these communities. Hence, a new data set was formed by excluding phytoplankton species, observed solely in the eutrophic or the oligotrophic habitat during the annual cycle, and thus containing individuals with inherent physiological adjustments that enable their propagation in broader areas. The computation of the similarity indices and the resultant classification patterns for the coastal area of Rhodes and the gulf of Gera are represented in Figure 3A and B, respectively. It can be seen that the grouping of the stations and the intra- or intergroup distances were almost identical to those derived from the whole data set. It seems possible that a compositional overlap above the level of 60–65% is indicative of environments with structural analogies, suitable for objective comparisons in the level of individual species. Contrarily, in the cases where the proportion of the common species is lower or there is an inconsistency among the results of this subset and those extracted from the whole data set, it would be safer to seek another control site in the neighborhood of the coastal stations. Furthermore, the following paragraph investigates an alternative approach to overcome the problem of the environmental "noise" in the spatial distribution of phytoplankton communities by exploiting species with significant

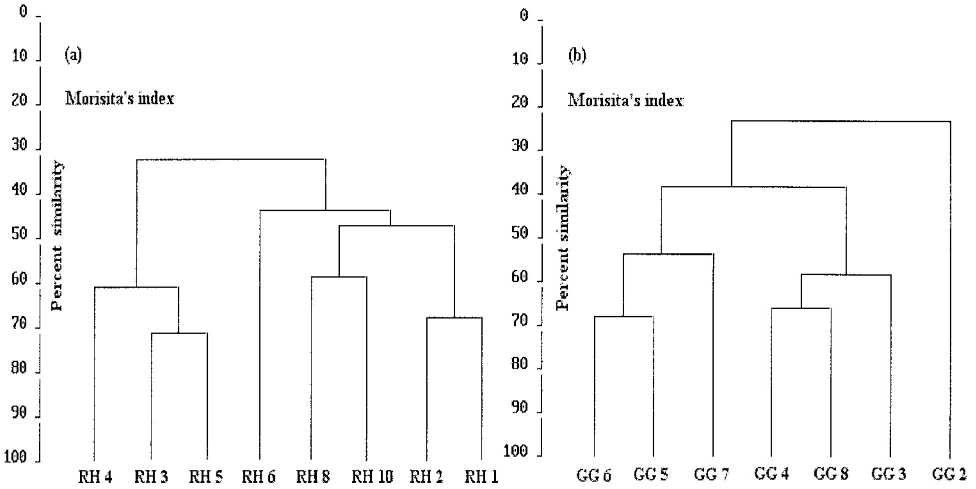

Figure 3. Grouping of (**a**) the nearshore stations of Rhodes and (**b**) the internal stations of the gulf of Gera based on the common phytoplankton species among the coastal area and the reference sites. The resemblance measure among the stations and the reference sites was Morisita's index.

growth rates and a permanent or a temporary behavioral adaptation in the nutrient enrichment of the coastal ecosystems.

Opportunistic Species

Fundamental concept. Nutrient accumulation in coastal waters leads to nonequilibrium processes at the phytoplankton community level producing patterns of abundance and species occurrences significantly different from those of the equilibrium situations (Harris 1986). Under such conditions, the communities are characterized by the presence of opportunistic species with an inherent capability to promptly react to environmental changes and show rapid growth rates until an upper limit is reached or until the excessive nutrient stock is exhausted (Sanford and Crawford 2000). Meanwhile, these dominant phytoplankton species co-exist with species that remain substantially unaffected from the external perturbations and present low levels of biomass. These rare species, resulting either from competitive exclusion or from brief and occasional growth potential, seem to be unsuitable for eutrophication studies (Allen and Star 1982, Cao and others 1998). Thus, the partitioning of the phytoplankton communities into the opportunistic and rare species categories and the combined use of the first category with the previous described similarity indices is likely to improve the resolution of this method.

The critical point of this approach is the selection of the criterion to distinguish between opportunistic and rare species. In the present work, the ratio of the total annual abundance of individual species to the respective number of observations was deemed more appropriate than using solely one of these two quantitative characteristics of the phytoplankton ecology. This criterion emphasizes not only the dominant species with a frequent occurrence over the annual cycle, but also species with perennation mechanisms that operate at seasonal cycles and temporarily constitute a significant fraction of the opportunistic part of communities (Gasol and others 1997). Furthermore, it was considered that since the high values of this ratio express mostly extreme environmental states, the detection procedure of the opportunistic species should handle them as "outliers". The discrimination of the outlying observations was based on the use of a simple algorithmic procedure known as the "Box and Whisker Plot" (Ott 1988). This method does not assume normality and can be used in nonsymmetrical distributions. A box plot can be easily constructed from the information referring to the quartiles. The second and third quartiles are graphically displayed as boxes while straight lines connect each box to the minimum and maximum value, respectively. Data points lying outside 1.5 times the interquartile range of the upper quartile were recorded as outliers.

Applications in the study areas. The frequency distributions of phytoplankton species in the study areas are presented in Figure 4. The construction of these profiles was obtained by summing the abundances of individual species from all the sampling units of the discharged sites of Gera [GG2]-[GG8] and Rhodes (all the stations except the oligotrophic sites [RH7] and [RH9] over the annual cycle). The distribution patterns are

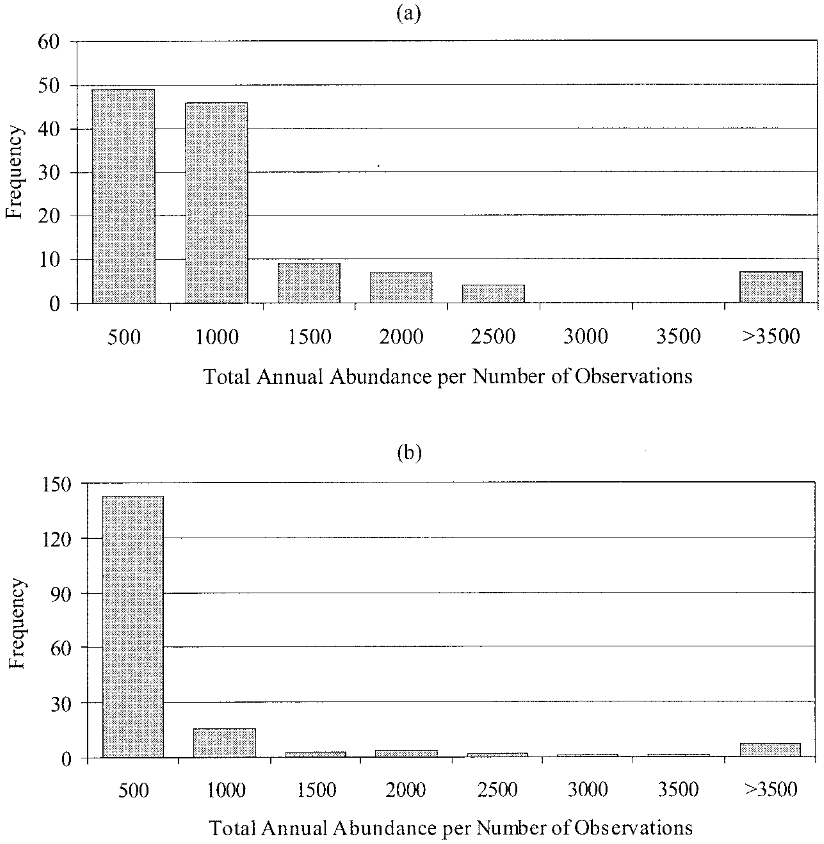

Figure 4. Frequency distribution of phytoplankton species (**a**) in the gulf of Gera (stations GG2–GG8) and (**b**) in the coastal area of the city of Rhodes (all the stations except the oligotrophic sites RH7 and RH9).

highly asymmetrical with a well-expressed skew to the right, indicating that (a) rare species constitute the major portion of these phytoplankton communities; and (b) there is a number of species loosely related to the central tendency of the studied populations (outliers). The extreme values detected by the Box and Whisker Plot are shown in Figure 5. It was an iterated process until outlying values could not be further detected. The number of opportunistic species was about 25% and 33% of the total number of species observed in the coastal area of Rhodes and the gulf of Gera, respectively. The highest number of outlying observations in the gulf of Gera should be attributed to its morphology, i.e., a shallow and semi-enclosed basin with limited exchanges with the open sea, constituting an unstable marine environment associated quite frequently with non-equilibrium processes due to the wider fluctuations of the physical (temperature, density, salinity) and chemical (nutrient accumulation) conditions.

The application of the similarity indices intended to measure the deviations of the opportunistic part of the enriched sites from the reference levels. Figure 6A illustrates the grouping of the internal stations of the gulf of Gera and the near shore stations of the coastal area of Rhodes. The resemblance measure among the stations and the control sites is Morisita's index, but similar inferences were extracted from the rest of the indices. In the case of Gera, the classification pattern of the stations was the same as the one observed with the whole body of data. The stations [GG5], [GG6], [GG7] formed a small cluster; a similar cluster was formed by the stations [GG3], [GG4] and [GG8], whereas both the groups differed significantly from the station [GG2] at the entrance of the gulf. However, it is evident that the clusters based on the outlying observations are more discrete and fuse at lower distances compared with the pattern illustrated in Figure 2B. A similar coherent grouping can be seen from the dendrogram of Rhodes (Figure 6B). Moreover, in this particular case the use of the opportunistic species has led to another interesting result for the station [RH6], which is no longer linked with the other stations that monitor waters used for swimming and other recreational activities. The distinct behavior of this site should be attributed mostly to a) the larger number of swimmers, resulting in the highest level of ammonium in the coastal area, and b) the proximity to the harbor, both

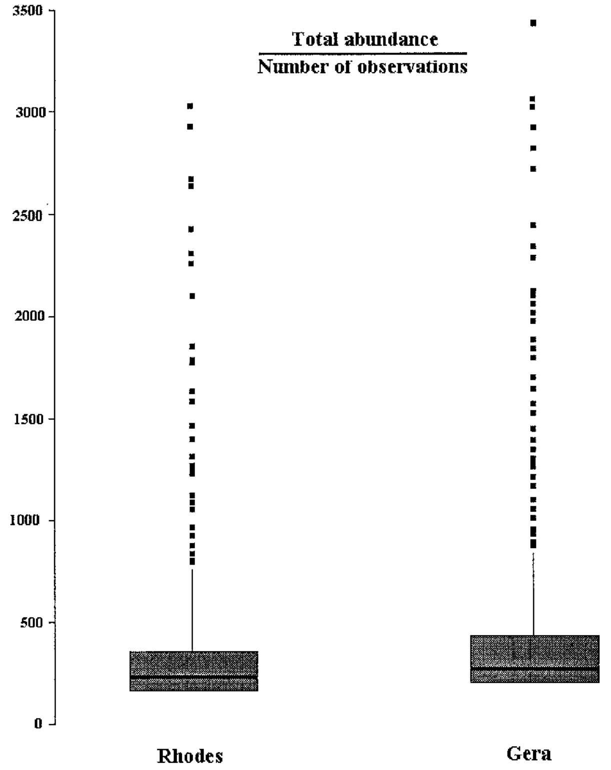

Figure 5. Box-and whisker plots for detecting opportunistic species: the procedure was reiterated until no further extreme values could be detected.

constituting stimulants for additional growth of the dominant fraction of phytoplankton community. Thus, the combined use of the similarity indices with the dominant phytoplankton species creates a sensitive technique that provides additional information about the structural shifts of the phytoplankton communities under external perturbations and emphasizes discontinuities in the mesotrophic levels.

Finally, the specific method can be proposed as a qualitative measure of the renewal processes of coastal marine ecosystems that regulate the residence time of excessive nutrient loads, especially in lagoons and gulfs. Figure 7 illustrates the differences in grouping of the internal stations of the gulf of Gera, between winter and summer. These classification patterns were based on a further separation of the set of opportunistic species into two subsets, consisting of their abundances during the stratified (April-October) and the nonstratified (November-March) period. In Figure 7a, the internal stations ([GG4]-[GG8]) show a significant homogeneity forming a cluster with a mean similarity level of 65%. This result is indicative of the insularity of the system during the winter, when the ambient temperature and the inflows of the cold-water masses of the runoff render this shallow gulf denser than the external system. Meanwhile, the inlet of the gulf has a screening role that limits the entrance of the oligotrophic waters of the Aegean Sea, a process that is associated with the group formed by the stations [GG2] and [GG3] at a mean distance of 55%. Conversely, the warm months of the year (April to October), the limiting physical factors are eliminated and the gulf is characterized by rapid exchanges with the open sea. The classification pattern of Figure 7b leads to three groups containing the stations of the northwestern part of the gulf [GG5], [GG6], [GG7], those of the southeastern part [GG3], [GG4], [GG8] and the station [GG2] at the entrance of the gulf. These groups are linked at higher distances indicating the importance of the advective and diffusion transport to the phytoplankton patterns, since these processes provide energy supplement to the community, increasing the spatial heterogeneity inside the gulf.

Discussion

The choice of an index for water quality studies should be the result of careful consideration and understanding of the natural mechanisms that cause the stress. In the case of eutrophication, it must be realized that the initial nutrient enrichment may cause an increase in diversity, but this can be at the expense of a shift in species composition of the community (Van Donk and others 1997). In the present work, considering the ambiguity that covers the spectrum of perturbation-diversity interactions, a new methodology is proposed focusing on the intra- and interspecific alteration of phytoplankton communities among sites with different levels of nutrient loading. The basic aim of this approach is the comparison among enriched sites (i.e., coastal areas) in terms of the compositional divergence of their phytoplankton communities from the baseline conditions. In striving for objectivity of these comparisons, the effects of spatial heterogeneity and seasonality are isolated using nearby unaffected areas and comparing sampling units that cover all of the annual cycle. Moreover, the variability of selective grazing pressures among sites with different trophic conditions and the effects of this variability on the interspecific abundance distributions is not excluded from the analysis, since the alterations in the phytoplankton-zooplankton relationships—usually weak in marine ecosystems (Micheli 1999)— were regarded as a sequential process of the eutrophication phenomenon that should be inherent in the results.

Nine common similarity indices, measuring the resemblance between enriched and control areas, were screened for their sensitivity to assess different levels of eutrophication. These indices varied in terms of a) the

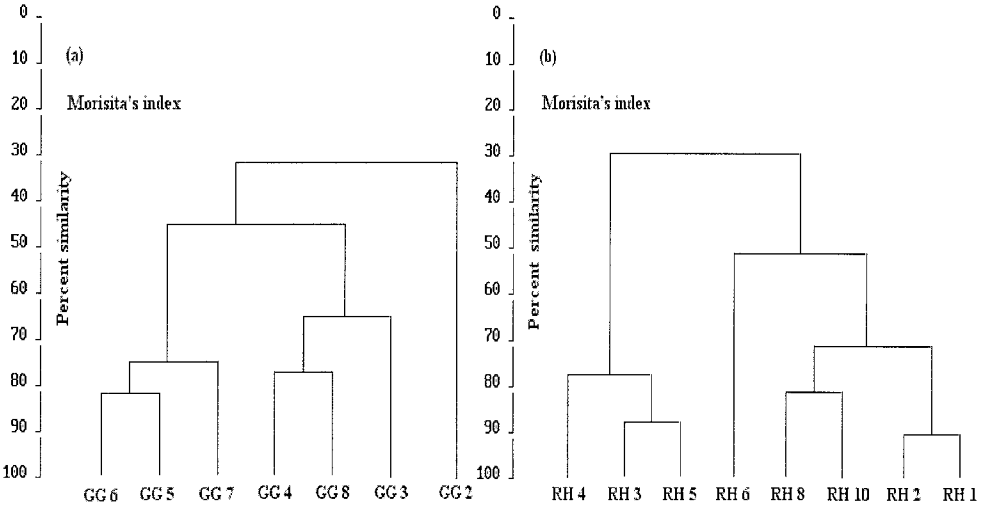

Figure 6. Grouping of (**a**) the internal stations of the gulf of Gera and (**b**) the nearshore stations of Rhodes based on the opportunistic phytoplankton species. The resemblance measure among the stations and the reference sites was Morisita's index.

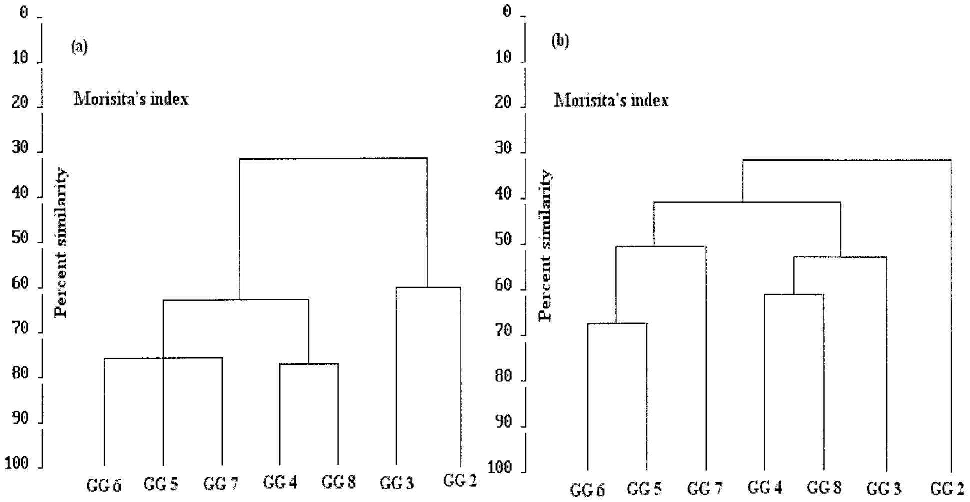

Figure 7. Grouping of the internal stations of the gulf of Gera, after the detection of opportunistic species, using numerical classification based on Morisita's index: (**a**) during the winter and (**b**) summer period.

assigned priorities in the deviations among the abundant (i.e., Pandeya's index) and rare (i.e., Camberra metric) species, b) the different (i.e., Ellenberg's index) or the same (Bray-Curtis' index) processing of the common and noncommon subcategories. Two phytoplankton data sets, one from an open coastal system and one from a semi-enclosed gulf, associated with different nutrient dynamics and circulation patterns were used for evaluating the consistency and potential extrapolation of these methods. Generally, all the similarity indices have led to clear-cut patterns and have stimulated rational hypotheses concerning the spatial variability of the study areas. In the case of Gera, the application of the similarity indices classified the internal stations into two groups representing the northwestern [GG5], [GG6], [GG7] and southeastern [GG3], [GG4], [GG8] part of the gulf, due to the differences of the nutrient discharges from the respective segments of the watershed in combination with the circulation pattern of the gulf that leads to an inadequate renewal of the seawater of the inner parts, especially during the periods of limited exchanges with the open sea (Arhonditsis and others 2000). In a similar way, the classification patterns of the coastal area of Rhodes indicated a clear distinction between polluted sites [RH3], [RH4], [RH5] near the harbor and the sites used for swimming and other recreational activities [RH1], [RH2], [RH6], [RH8], [RH10]. On the other hand, the application of evenness, diversity and dominance indices in the same data sets was characterized by reduced sensitivity in discriminating among different eutrophication levels and resulted in inconsistent and confusing patterns (Karydis and Tsirtsis 1996, Tsirtsis and Karydis 1998, Arhonditsis 1998).

These indices exclusively express the richness and variety of natural ecological communities (diversity indices), the equitability of species abundance in the sample/community (evenness), or emphasize the role of the most important species (dominance) (Washington 1984). Apparently, the sole use of one of these properties as a tracer for elucidating the phytoplankton community responses under conditions of nutrient enrichment is not a robust and consistent methodological tool. In contrast, the multi-component nature of the similarity indices formulations, incorporating most of the above information along with the divergence of these structural characteristics from the baseline conditions, enables the identification of even small quantitative or qualitative differences in the trophic status.

The structure of the oligotrophic environment and the extent to which it regulates the subsequent habitat, an implicit source of bias for the results, was also examined by the study. It was deemed that the information concerning the fraction of the non-occurring species between two neighboring sites is more liable to sampling and counting problems, whereas its magnitude is mostly associated with ecological conditions and physical processes that have little to do with eutrophication processes and anthropogenous disturbances (Harris 1986). Thus, the validity of the method and the suitability of the control sites to provide the baseline information for the phytoplankton community structure can be sought in the overlapping fraction of species among the different areas. This procedure excludes from the analysis a useful part of the community, such as the species that have different levels of adaptation (i.e., competitive exclusion) under low and high nutrient concentrations. However, the cost of loosing this piece of information is compensated by focusing on the abundance patterns of species with a greater resilience in the spatial variability of the physical forcing and thus partly removing the environmental "noise", as outlined for this particular study.

The classification patterns of this data set were almost similar to those that resulted from the whole body of data, possibly due to the significant proportion of the compositional overlap (>65%) among the study communities, confirming the existence of the previously described spatial trends. Hence, the proportion of the common species among communities and the extent to which the information extracted from this fraction is distorted when analyzing the overall communities, can form an objectivity criterion for comparisons that seek out dissimilarities caused from eutrophication processes.

A further improvement of the specific method was attempted by exploiting the abundances of the species that are favored mostly under conditions of excessive nutrient loading, the so-called opportunistic species. The criterion for their identification was the ratio of the total annual abundance of individual species to the respective number of observations. The non-parametric "Box and Whisker Plot" was deemed appropriate for detecting the dominant species (Karydis 1994), since the basic assumption of this work was the idea that the opportunistic species represent the most flexible fraction of phytoplankton communities and should be considered as outliers induced from extreme environmental states. The use of the abundances of these species has improved the results, emphasizing discrete sites and smoothing out differences between similar sites. Furthermore, in the data set of Rhodes, the specific method showed an increased sensitivity in detecting structural dissimilarities in the mesotrophic level, thus, the hypotheses involving the trophic status of the station RH6 that seems to lie between the polluted and the waters used for swimming, possibly due to its vicinity to urban areas. This property of the method is essential for coastal management since mesotrophic waters form the early warning systems for environmental quality assessment (Karydis 1992).

Finally, there is a possibility that the correspondence between opportunists and outliers and consequently the application of the "Box and Whisker Plot" will not be so effective when the frequency distribution of species abundance is symmetrical and not so skewed as in these particular cases. Under such conditions, the discrimination of the opportunistic part will be determined by introducing in the analysis of phytoplankton communities the concepts of central and marginal populations (Emlen 1984). The development of an objective technique that distinguishes the two categories for different locations and physical conditions constitutes an aim for on-going research.

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