Evanthia Lekka[a], Ifigenia Kagalou[b], Maria Lazaridou-Dimitriadou[a], Triantafilos Albanis[c], Vasileios Dakos[a], Dimitra Lambropoulou[c], Vasilis Sakkas[c]

[a] Department of Zoology, School of Biology, Faculty of Sciences, Aristotle University of Thessaloniki, 54006, Thessaloniki, Greece

[b] T.E.I. of Epirus, Department of Ichthyology and Fisheries, Eirinis and Filias 1, 46100, Igoumenitsa, Greece

[c] Laboratory of Industrial Chemistry, School of Chemistry, Faculty of Sciences, University of Ioannina, 45110, Ioannina, Greece

Assessment of the Water and Habitat Quality of a Mediterranean River (Kalamas, Epirus, Hellas), in Accordance with the EU Water Framework Directive

In the present study, the water quality of Kalamas river (NW Greece) was evaluated using physicochemical and hydromorphological parameters and benthic macroinvertebrates. Statistical analyses (Cluster and FUZZY analyses) were performed and two biotic scores (BMWP' and HS) were used in order to classify the sites according to water quality. Kalamas river appeared to have excellent tomoderate water quality at all sampling sites except one (close to the delta area) which was "fairly or significantly polluted". During the low flow season water quality appeared poorer than during the high flow season. The ecological parameters (hydromorphological, chemical, and biological) used for this integrated approach are the ones proposed by the New Water Directive 2000/60 EC for an efficient surveying monitoring of running waters.

Einschätzung der Wasser- und Habitat-Beschaffenheit eines mediterranen Flusses (Kalamas, Epirus, Hellas) in Übereinstimmung mit der EU-Wasserrahmenrichtlinie

Die Wasserbeschaffenheit des Kalamas River (Nordwest-Griechenland) wird auf der Basis physikalisch-chemischer und hydromorphologischer Parameter und der benthischen Makrofauna dargestellt. Statistische Analysen (Cluster- und FUZZY-Analysen) und zwei biotische Indices (NMWP und HS) werden zur Klassifizierung der Probestellen hinsichtlich der Wasserbeschaffenheit genutzt. Danach weist der Kalamas River an allen Stellen eine sehr gute bis gute Wasserbeschaffenheit auf, ausgenommen der unterste, nahe dem Delta gelegene Abschnitt, der deutlich oder signifikant verunreinigt ist. Die Gewässerbeschaffenheit scheint während der Niedrigwasserperiode schlechter zu sein als zu Zeiten höherer Durchflüsse. Die ökologischen Parameter (hydromorphologische, chemische und biologische), die für diese integrierte Bewertung verwendet wurden, entsprechen denjenigen, die von der Wasserrahmenrichtlinie 2000/60 der EU für ein effizientes Monitoring der Fließgewässer vorgeschlagen werden.

Keywords: Benthic Macroinvertebrates, Biomonitoring, River Habitat Survey

Schlagwörter: Benthische Makrozoen, Biomonitoring, Monitoring, Gewässerhabitat

Research Paper

Correspondence: M. Lazaridou-Dimitriadou, E-mail: mlazarid@bio.auth.gr

DOI 10.1002/aheh.200300528

1 Introduction

Rivers are important pathways for the flow of energy, matter, and organisms through the landscape. A wide range of human activities at the catchment's areas may lead to environmental deterioration of river waters. However, the assessment of the changes in river communities as the result of the impact of pollution is a particularly interesting issue within the framework of aquatic ecology [1].

Studies from Mediterranean rivers are scarce and focus mainly on France and on the Iberian Peninsula [2, 3]. Greek river systems, however, as well as some other Mediterranean systems, are characterized by an extended summer period of dryness, during which intense land use practices close or in the drainage basin influence the river biota (macrophytes, benthic invertebrates etc).

According to the new "Water Framework Directive" (2000/60) of the EU [4], all member states are obliged to establish national monitoring systems and determine the 'ecological status' of water quality, referring to the biological elements of the ecosystem as well as to the river habitat quality. Biomonitoring is already widespread since it provides information on the water quality and ecology [5–7]. Biomonitoring includes both sublethal changes at the cellular or tissue level [8, 9] and changes in community structure [10]. The use of changes in community structure commonly involves benthic invertebrates, since they are considered to be the most appropriate biotic indicator of water quality in EU countries, including Greece [5, 6, 11]. Additionally, the assessment of the river habitat quality is essential, since it may affect the structure and composition of the resident benthic communities [12]. Consequently, an integrated estimation of the water quality requires the combination of biological, physicochemical, and hydromorphological data.

In the present study, the water and habitat quality of the river Kalamas was estimated using only benthic invertebrates (and not fish, macrophytes, and algae as the WFD proposes, since the benthic invertebrates are considered as the most effective bioindicators for organic pollution [6]), hydromorphological parameters, and chemical quality data. The ecological parameters (hydromorphological, chemical and benthic macroinvertebrates) used for this integrated approach are the ones proposed by the new Water Directive 2000/60 EC for an efficient surveying monitoring of running waters.

This river Kalamas was chosen because it has a typical Mediterranean hydrological regime and it is one of the most important aquatic systems of the western Greece, entirely within Greek territory. The protection and management of Kalamas waters are of great economic and ecological importance and should be based on an integrated environmental approach.

2 Materials and methods

2.1 Study area

Kalamas is a river with a length of 113 km, originating from the central of Epirus and discharging into the Ionian Sea (Fig. 1). Its drainage area is 1747 km^2. It can be considered as a middle-size river with seasonal changes in water level [13]. Water from Pamvotis lake is led to the Kalamas river through a narrow channel named Lapsista, which receives urban and industrial wastes [14, 15]. The volume of the outflow through this connection fluctuates depending on the water level of the lake and usually discontinues during summer. The maximum water discharge of Kalamas is 54 m^3/s in winter and the minimum 32 m^3/s in summer at site Soulopoulo (see below for the description of the sites). The climate of the study area is Mediterranean with a pronounced summer drought and a mild winter. Average annual rainfall is about 1100 mm and mean air temperature is 17.4 °C. Spring and late autumn are the most rainy seasons [13]. In the catchment area corn, grains, and vegetables cover 20...30 % of the agricultural cultivations. Although protected by national and international laws (its delta is characterized as Important Bird Area and proposed to the "NATURA 2000" network), the river receives urban effluents, treated domestic

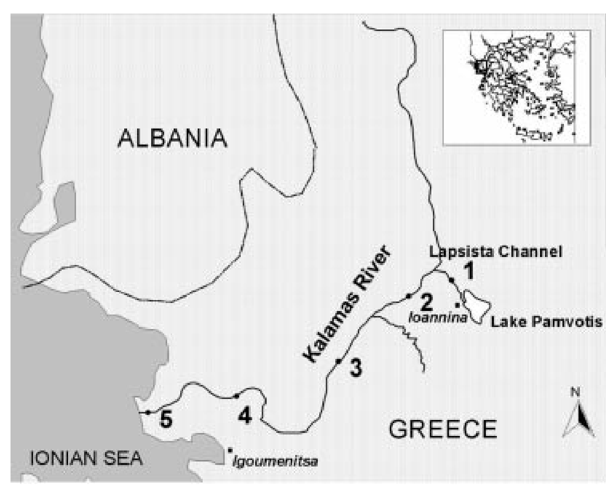

Fig. 1: Map of the study area showing Kalamas river and the sampling sites Vrontismeni (1), Soulopoulo (2), Vrosina (3), Neraida (4), and Ragio (5).

Karte des Untersuchungsgebietes mit den Probenahmestellen am Kalamas River: Vrontismeni (1), Soulopoulo (2), Vrosina (3), Neraida (4) und Ragio (5).

as well as untreated effluents from many semi-urban settlements, small-scale industrial discharges, and surface runoff from agricultural land. Point sources of pollution are scattered along the whole length of the river, whereas along the lowland part of the river intensive agricultural activities take place.

The physicochemical characteristics, as well as pesticide residues and heavy metal levels have been studied by Albanis et al. [14], Stalikas et al. [15], Mane et al. [16]. Pesticides in the river are higher than those detected in the Lapsista canal [14]. No studies concerning biological features have been done, except one concerning chlorophyll variation and macrophyte abundance [17].

2.2 Methodology

Samples of benthic macroinvertebrates were taken at monthly intervals, from March 2000 to February 2001 at five sampling stations (sites). Site Vrontismeni was situated 8 km downstream the springs of Kalamas river, site Ragio close to the effluents of Kalamas river into the Ionian Sea and the rest three sites were situated along Kalamas river, in relation to point and non-point sources of pollution (Table 1).

The time-schedule of sampling as well as the sampling stations have been established in the framework of an EU project concerning the monitoring and management of Kalamas river (LIFE99ENV/G12/000557, www.uoi.gr/life).

Two samples (replicates) of benthic macroinvertebrates were taken at each one sampling station with the 3-minute kick-sweep method [11], using a standard pond net (surface 575 cm^2, mesh size 900 μm, depth 27.5 cm). The samples were placed in plastic bottles and preserved in 4% formaldehyde. On return to the laboratory, the animals were sorted using a 0.5 mm mesh sieve. Identification of the invertebrates was made to the lowest possible taxonomic level (genus level, except for Platyelminthes and Diptera, which were identified to the family level, and Lepidoptera, Heteroptera, Oligochaeta, Hirudinea, and Decapoda-Crustacea which were identified to order or class level). More information about the detailed macroinvertebrate fauna found at each site is available from the authors.

Surface water samples were taken from the same sampling stations, using polypropylene bottles. The samples were analyzed in the laboratory according to APHA methods [18]. The following parameters were examined: temperature, conductivity, total dissolved solids (TDS), pH and dissolved oxygen (in situ measurements using electrode probes WTW type), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), nitrate, nitrite, ammonia, and phosphate. Substrate composition was also estimated visually by percentage occurrence of the following particle categories using the Wentworth scale: clay/silt (< 0.0625 mm), sand (0.0625...2 mm), gravel (2...4 mm), pebbles (4...16 mm), cobbles (16...256 mm), boulders (> 256 mm).

Table 1: Physical characteristics, human activities, and sources of pollution at the 5 sites of Kalamas river (Vrontismeni, Soulopoulo, Vrosina, Neraida, Ragio) during the study period.

Physikalische Charakteristika, Aktivitäten des Menschen und Belastungsquellen an den fünf Untersuchungsstellen des Kalamas River (Vrontismeni, Soulopoulo, Vrosina, Neraida, Ragio) während der Untersuchungsperiode.

	Distance from source km	Altitude m	Slope m/km	Sources of pollution	Human modifications
Vrontismeni	8	390	2.5	1, 3	B, C
Soulopoulo	29	160	1.724	4	B
Vrosina	46.5	100	6.67	1, 2, 3	A, C
Neraida	70	40	5.29	1, 2	A
Ragio	106.5	20	3.66	1, 2	D

Sources of pollution:
1: Agricultural fields
2: Livestock farm
3: Wastes (soil/liquid) from small industries or built-up areas
4: By-products of the bioremediation of Ioannina city

Human modifications:
A: Sand extraction
B: Water abstraction by pumping
C: Irrigation canals
D: Dam

2.3 Biotic indices and scores

The Iberian BMWP (BMWP') [19] and the Hellenic Score (HS) [20] were applied to the macroinvertebrate data. The BMWP' index is a modification of the English BMWP adapted for the Iberian Peninsula. The HS was created according to BMWP' (after the addition of a number of families and the reassessment of all the family scores according to the specific conditions of Hellenic rivers) and it takes into consideration the relative abundance of the taxa found.

2.4 Statistical analyses

For the classification of the 60 samples FUZZY clustering and PRIMER statistical analysis were used. FUZZY clustering analysis [21] was performed, in order to obtain both ordination and classification of the samples. FUZZY technique, which is an extension of TWINSPAN [22], produces clusters of the assemblages of benthic macroinvertebrates in each sample, according to their membership value. The numbers of FUZZY clusters are selected according to the higher partition coefficient. FUZZY analysis is more suitable for the description of riverine ecological communities than TWINSPAN, since it is not a hierarchical method.

PRIMER analysis [23] produces a similarity dendrogram of sampling stations based on the presence-absence and the abundance of benthic macroinvertebrate taxa. It is a hierarchical method, which measures the similarity of stations using the Bray-Curtis similarity index. SIMPER analysis explains which families of macroinvertebrates contribute to the similarity or dissimilarity between the groups of PRIMER.

2.5 Habitat quality

The recording of the physical structure of each site was conducted in May 2000 along with the physicochemical and biological samplings. The recording of the physical structure at each site was based on the River Habitat Survey (RHS) [24] at each sampling site on standard 500 m length river stretch in order to mark its efficiency in the Greek rivers. It involved a systematic collection of features associated with the physical structure of the watercourse recorded at 10 spot-checks in 50 m intervals. Such features were substrate, flow, vegetation types, erosion, and deposition elements in the channel, morphological and vegetation structure on the banks, and land use in the adjacent river corridor. Modifications to channel and bank structure were also recorded. For a detailed description see also [25, 26]. Data were computerized with the help of RHS 3.2 database. The habitat quality of each site was assessed by using the Habitat Quality Assessment Score (HQA), which is based on the diversity of the physical features recorded and mentioned above. Artificial modification was estimated by a second scoring system, the Habitat-Modification Score (HMS) that gives "penalty" points to different types of alteration. Sites were divided in five categories according to their HMS scores (Table 2). The comparison of the habitat quality of the 5 studied sites was achieved by performing a principal component analysis (PCA) of an inventory of sites already surveyed along Greek rivers, in order to determine sites of similar type. For this determination the PCA takes into account the slope and altitude of the sites, their distance from the source and the altitude of the source. The habitat quality of the 5 sites was judged by comparing their HQA scores with the ones of the similar sites.

Table 2: Overview of how the Habitat Modification Categories are derived from the Habitat Modification Scores.

Übersicht zur Ableitung der Kategorien der Habitat-Modifikation aus dem Index der Habitat-Modifikation (HMS).

Habitat Modification Score (HMS)	Habitat Modification Categories
0...2	Semi-natural
3...8	Predominantly unmodified
9...20	Obviously unmodified
21...44	Significantly modified
45...	Severely modified

3 Results

3.1 Physicochemical parameters

Temperature varied ranging from 6.5 °C (Soulopoulo in February and December) and 21.7 °C (Ragio in July) (Table 3), while pH ranged between 7.59 (Ragio in August) and 8.14 (Vrosina in October) without significant changes between the sampling points. The highest value of conductivity was recorded at the station Vrosntismeni in August (815 μS/cm) and the lowest at station Neraida in September (120 μS/cm). The range of total dissolved solids was from 60 mg/L (Neraida in August) to 467 mg/L (Soulopoulo in November) (Table 3).

The nitrate-nitrogen concentrations ranged between 0.05 mg/L and 2.67 mg/L (Fig. 2a). Ammonium slightly fluctuated being always lower than the detection limit. Higher values of nitrite-nitrogen and phosphates were observed in March, April, or May (Fig. 2b, 2c). In most samples phosphate concentrations were higher than 500 μg/L, which is considered as the lower limit for river waters to pose a risk of eutrophication

Table 3: Maximum (max.), minimum (min.), and average (aver.) values of temperature (Temp), pH, conductivity (Cond.), total dissolved solids (TDS), and dissolved oxygen (DO) in Kalamas sites during 2000. The site at where the max. or min. concentration was recorded is presented in parenthesis. Vt: Vrotnismeni, S: Soulopoulo, Vr: Vrosina, N: Neraida, R: Ragio. 3 samples were taken for each site.

Maximale (max.), minimale (min.) und mittlere (aver.) Werte für Wassertemperatur, pH-Wert, elektrische Leitfähigkeit, Gesamtsalzgehalt und gelösten Sauerstoff (DO) an den Probenahmestellen im Kalamas River im Jahr 2000. In Klammern die Abkürzung jener Probenahmestelle, an welcher der Extremwert auftrat. Vt: Vrotnismeni, S: Soulopoulo, Vr: Vrosina, N: Neraida, R: Ragio. Drei Messungen je Probenahmestelle.

Month		Temp °C	pH	Cond. μS/cm	TDS mg/L	DO mg/L
Jan	max.	8.5(R)	7.95(N)	702(S)	313(S)	16.4(S)
	min.	6.7(S)	7.85(S)	457(VT)	203(Vt)	14.5(R)
	aver.	7.2	7.82	548	243	15.5
Feb	max.	8.8(R)	7.91(Vr)	620(S)	275(S)	17.4(R)
	min.	6.5(S)	7.72(N)	456(N)	163(Vt)	15.8(S)
	aver.	7.5	7.83	549	220	16.5
Mar	max.	13.1(R)	7.95(Vr)	650(S)	357(S)	17.6(S)
	min.	9.0(Vt)	7.77(Vt)	243(N)	(Vt)	17.3(S3)
	aver.	1038	7.88	467	305	17.4
Apr	max.	14.0(R)	7.98(Vr)	710(S)	360(S)	17.5(S)
	min.	11.7(S)	7.70(Vt)	270(N)	220(Vt)	15.8(R)
	aver.	12.2	7.88	503	275	16.5
May	max.	18.8(R)	8.67(Vr)	731(S)	351(S)	14.6(S)
	min.	15(N)	7.09(Vt)	541(Vt)	236(R)	13.6(N)
	aver.	17.3	7.91	612	293	14
Jun	max.	18.5(R)	8.01(Vr)	815(Vt)	459(Vt)	14.5(S)
	min.	14.8(S)	7.72(Vt)	554(N)	291(R)	10(R)
	aver.	16.2	7.86	686	362	12.3
Jul	max.	21.7(R)	7.88(S)	320(Vt)	124(Vt)	12.5(S)
	min.	17.5(S3)	7.84(R)	179(N)	76(N)	9.8(R)
	aver.	18.8	7.86	221	92	10.8
Aug	max.	20.1(R)	8.03(Vr)	266(Vt)	107(Vt)	16.8(S)
	min.	16.8(S)	7.59(R)	150(S3)	60(N)	10.2(Vt)
	aver.	17.8	7.91	190	79	13.4
Sep	max.	15.7(N)	7.99(Vr)	165(S)	100(S)	16.4(S)
	min.	13.1(S)	7.65(Vt)	120(N)	70(N)	11.0(R)
	aver.	14.5	7.88	143	86	13.7
Oct	max.	13.2(N)	8.14(Vr)	654(S)	267(N)	17.6(S)
	min.	9.5(S)	7.84(Vt)	312(N)	198(R)	13.2(R)
	aver.	11.1	8	448	215	15.1
Nov	max.	9.5(R)	8.13(Vr)	705(S)	467(S)	17.2(S)
	min.	6.8(S)	7.91(Vt)	479(N)	326(N)	15(R)
	aver.	8.9	8.04	564	380	15.8
Dec	max.	8.1(R)	8.09(Vr)	513(S)	345(S)	16.9(S)
	min.	6.5(S)	7.85(Vt)	287(N)	231(N)	14.4(R)
	aver.	7	7.94	376	278	15.7

[27]. Organic load, expressed by BOD_5 and COD measures, presented higher values at the lowland stations and especially during the warm dry period (Fig. 2d, 2e).

The sites Vrontismeni, Soulopoulo, Vrosina, and Neraida were characterized by a diverse substrate of mainly coarse material (boulders, cobbles, pebbles, gravel) (Fig. 3). On the

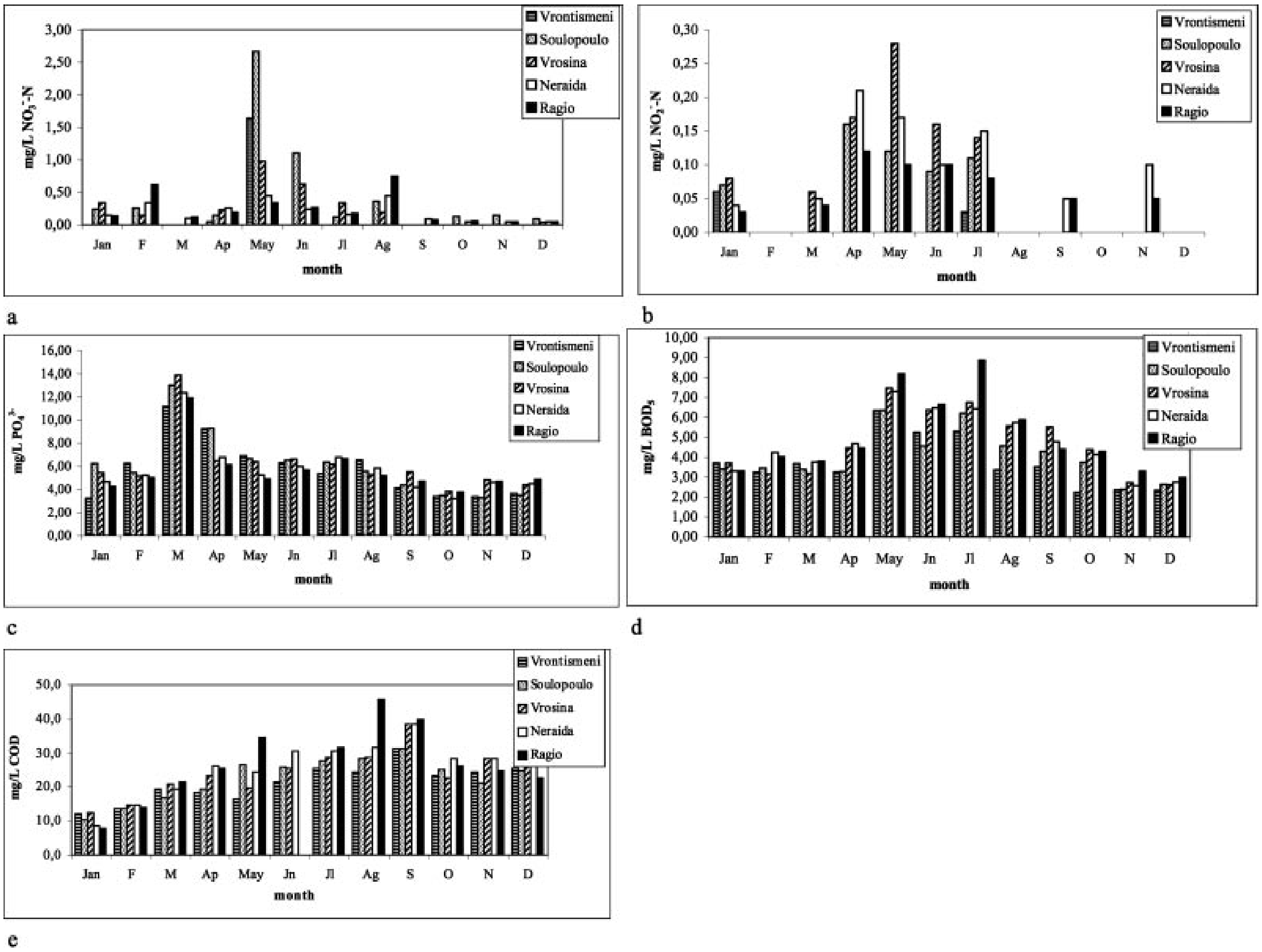

Fig. 2: Seasonal variation of physicochemical parameters NO_3^- (a), NO_2^- (b), PO_4^{3-} (c), BOD_5 (d), COD (e) at Kalamas sites (Vrontismeni, Soulopoulo, Vrosina, Neraida, Ragio) during 2000.

Saisonale Variabilität der physikalisch-chemischen Parameter NO_3^- (a), NO_2^- (b), PO_4^{3-} (c), BOD_5 (d), COD (e) an den Probenahmestellen des Kalamas River (Vrontismeni, Soulopoulo, Vrosina, Neraida, Ragio) im Jahr 2000.

contrary, at Ragio site the only type of substrate found was clay-silt during the whole year. In July, excluding the absence of gravel from all sites, no significant difference was found regarding their substrate composition during the year.

3.2 Benthic macroinvertebrates

Thirty four thousand and six hundred individuals were sampled in the 5 sites (60 samples in total) during March 2000 – February 2001 and seventy (70) benthic macroinvertebrates taxa were identified during the present study. The dominant taxa were: *Hydropsyche* spp. and *Agapetus* spp. (Trichoptera), Chironomidae, Ceratopogonidae, Athericidae (Diptera*), Ephemera* spp.*, Caenis* spp., *Ephemerella* spp., *Baetis* spp. (Ephemeroptera), *Leuctra* spp., Perlidae (Plecoptera), Elmidae (Coleoptera), and Oligochaeta.

Each sampling site showed biological patchiness, with different community distributions (Table 4). Trichoptera were recorded in all sampling sites having a higher abundance in the upstream sites (15.59% at Vrontismeni and 33.53% at Soulopoulo of the total taxa found per site). Among the downstream sites Diptera appeared to have a remarkable contribution to the total abundance. Ephemeroptera were more diverse and they were found in all upstream and downstream sites, contributing between 52.13% (Vrontismeni) and 14.75% (Ragio) of the total taxa found per site. Plecoptera were recorded in all sites except for Ragio. Oligochaeta were present at all sampling sites, but mainly at Vrosina (22.35%).

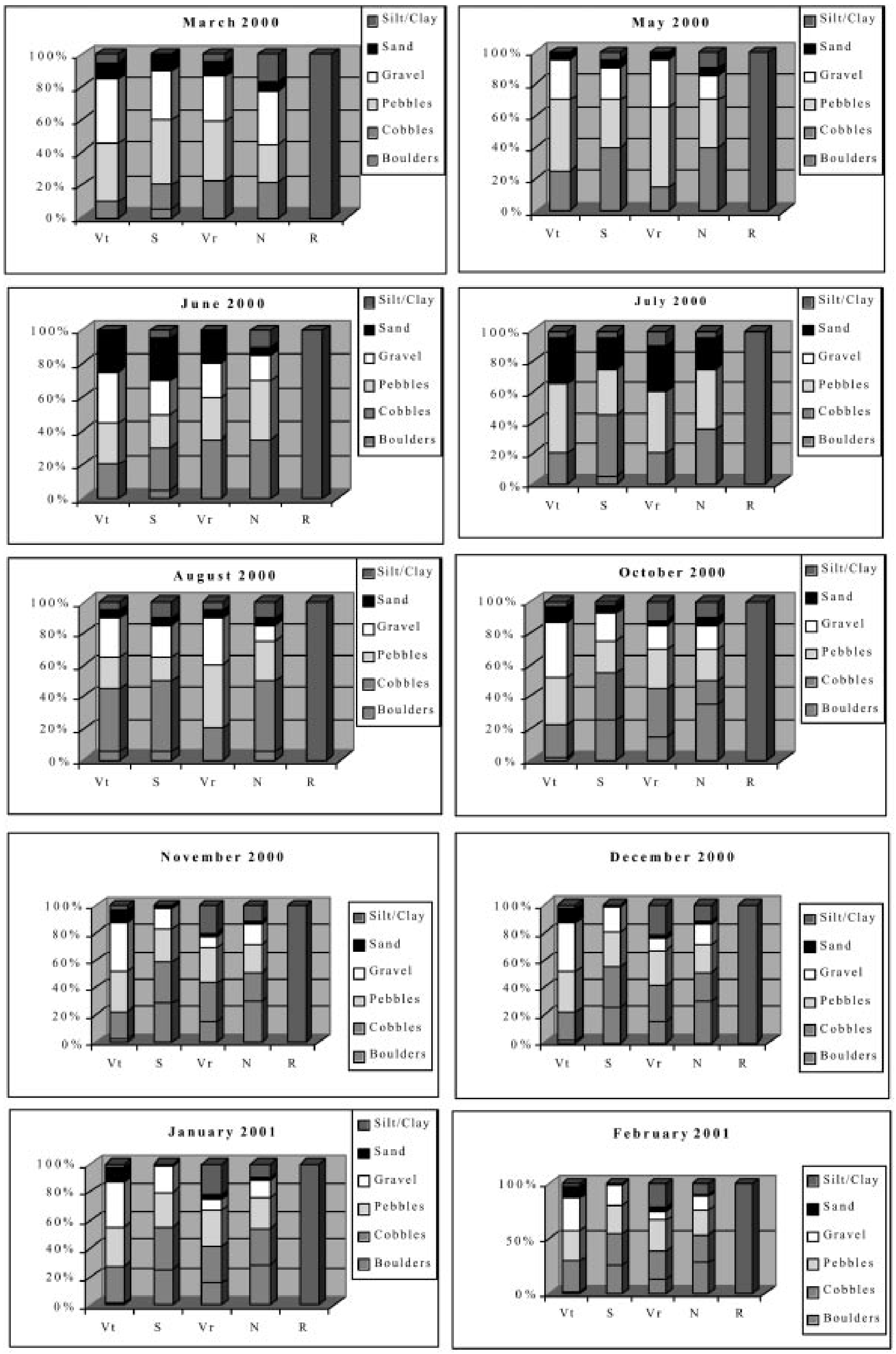

Fig. 3: Percent occurrence of substrate particle categories at Vrontismeni (Vt), Soulopoulo (S), Vrosina (Vr), Neraida (N), and Ragio (R) sites of Kalamas river during the sampling period 2000–2001 (no data are available for the months April and September 2000).

Prozentuales Vorkommen der Kategorien von Substratpartikeln an den Probenahmestellen Vrontismeni (Vt), Soulopoulo (S), Vrosina (Vr), Neraida (N) und Ragio (R) des Kalamas River im Untersuchungszeitraum 2000–2001 (Daten für April und September 2000 liegen nicht vor).

Table 4: Distribution (in %) of benthic macroinvertebrates classes, in each of the 5 sampling site of Kalamas river during the sampling period 2000−2001.

Prozentuale Verteilung der benthischen Makroinvertebraten an jeder der fünf Probenahmestellen des Kalamas River im Untersuchungszeitraum 2000−2001.

Phylum	Class/Order	Vrontismeni	Soulopoulo	Vrosina	Neraida	Ragio
Arthropoda	Trichoptera	15.59	33.53	3.86	9.64	3.69
	Diptera	15.53	15.10	39.63	46.43	70.97
	Ephemeroptera	52.13	34.45	23.26	31.59	14.75
	Plecoptera	5.62	5.34	4.53	1.88	0.00
	Odonata	0.17	0.20	0.71	0.78	0.23
	Coleoptera	6.77	7.43	1.47	3.95	0.08
	Heteroptera	0.01	0.01	0.00	0.00	0.08
	Lepidoptera	0.50	0.00	0.00	0.09	0.00
	Decapoda	0.07	0.00	0.00	0.00	0.00
	Amphipoda	0.03	0.24	0.11	0.08	0.00
Mollusca	Gastropoda	2.03	0.37	0.45	0.15	0.00
Annelida	Hirudinea	0.00	0.03	0.15	0.00	0.00
	Oligochaeta	0.49	1.75	22.35	3.36	7.22
Platyelminthes		0.00	0.69	0.02	0.00	0.00
Pupae		1.06	0.86	3.45	2.05	3.00

Concerning the low-abundance taxa, Coleoptera and Odonata were present at all sampling sites, while the rest of the taxa (Amphipoda, Gastropoda, Lepidoptera, Hirudinea, Platyelminthes, Heteroptera, and Decapoda) never constituted more than 3% of the benthic fauna at any site.

3.3 Biotic indices and scores

Table 5 presents the classification of the sites according to BMWP' and their scores according to the Hellenic Score (HS-Hellenic BMWP′). Generally, BMWP'score and HS did not present differences in describing the water quality of Kalamas sites. Some minor differences (mainly at Ragio site) can be attributed to the addition of a number of families and the reassessment of all the family scores in HS. BMWP' showed a poorer water quality during the months March, April, May, December, January, and February, indicating a temperature based variation, with higher values during the warmer (low-flow) months. Ragio site was categorized in class IV or V throughout the year, except in April (Class III). Soulopoulo was the only site to be categorized in class I during all year, except in September, when the water quality at all sites seemed to deteriorate. Soulopoulo presented the highest HS scores among all sites during the whole year (Table 5).

3.4 Statistical analyses

The 60 samples that were used for statistical analyses (abundance data were transformed into log (x+1)) were grouped by FUZZY analysis in three clusters (Fig. 4); the first group consists of all Ragio samples except the one of April, which belongs to the second group. The second group consists of the rest of the samples apart from the sample Sep-Soul, which comprises the third group.

PRIMER multivariate statistical package clustered the samples (the abundance data were transformed into log (x+1)) into 3 groups as well (at the level of 81.71%, Fig. 5):

a) the first group consists of sample Sep-Ragio by itself (average dissimilarity 97.52%),
b) the second group consists of the rest Ragio samples (apart from April), together with site Vrosina in August, September, and December (average dissimilarity 81.71%),
c) and in the third group belong the remaining samples clustered into several subgroups.

3.5 River habitat survey

The smooth relief and the small slope of all sites were depicted on the predominant smooth flow recorded at all sites.

Table 5: Values of BMWP' (Iberian BMWP) and HS (Hellenic Score – Hellenic BMWP) applied to the sites of Kalamas river during the sampling period 2000–2001. Mar: March, Apr: April, May: May, Jun: June, Jul: July, Aug: August, Sep: September, Oct: October, Nov: November, Dec: December, Jan: January, Feb: February, Vt: Vrontismeni, S: Soulopoulo, Vr: Vrosina, N: Neraida, R: Ragio.

Monatliche Werte des BMWP (Iberian BMWP) und HS (Hellenic Score – Hellenic BMWP) im Untersuchungszeitraum 2000–2001 für die Probenahmestellen des Kalamas River. Vt: Vrotnismeni, S: Soulopoulo, Vr: Vrosina, N: Neraida, R: Ragio.

BMWP' Month – Sites	Score	Class	Interpretation of Water quality	HS HBMWP
Mar – Vt	70	II	Evidence of contamination (Good)	708
Mar – S	112	I	Not contaminated (Excellent)	1233
Mar – Vr	96	II	Not contaminated (Excellent)	968
Mar – N	123	I	Not contaminated (Excellent)	1206
Mar – R	1	V	Significantly contaminated (Very Poor)	20
Apr – Vt	70	II	Evidence of contamination (Good)	701
Apr – S	102	I	Not contaminated (Excellent)	1069
Apr – Vr	80	II	Evidence of contamination (Good)	782
Apr – N	81	II	Evidence of contamination (Good)	851
Apr – R	56	III	Contaminated (Moderate)	543
May – Vt	86	II	Evidence of contamination (Good)	927
May – S	153	I	Not contaminated (Excellent)	1317
May – Vr	134	I	Not contaminated (Excellent)	1249
May – N	99	II	Evidence of contamination (Good)	1017
May – R	7	V	Significantly contaminated (Very Poor)	109
Jun – Vt	155	I	Not contaminated (Excellent)	1599
Jun – S	194	I	Not contaminated (Excellent)	1965
Jun – Vr	168	I	Not contaminated (Excellent)	1534
Jun – N	99	II	Evidence of contamination (Good)	1033
Jun – R	28	IV	Fairly contaminated (Poor)	275
Jul – Vt	133	I	Not contaminated (Excellent)	1420
Jul – S	190	I	Not contaminated (Excellent)	1874
Jul – Vr	147	I	Not contaminated (Excellent)	1371
Jul – N	105	I	Not contaminated (Excellent)	1041
Jul – R	14	V	Significantly contaminated (Very Poor)	144
Aug – Vt	126	I	Not contaminated (Excellent)	1237
Aug – S	165	I	Not contaminated (Excellent)	1568
Aug – Vr	90	II	Evidence of contamination (Good)	869
Aug – N	105	I	Not contaminated (Excellent)	1097
Aug – R	15	V	Significantly contaminated (Very Poor)	164
Sep – Vt	5	V	Significantly contaminated (Very Poor)	67
Sep – S	69	II	Evidence of contamination (Good)	754
Sep – Vr	24	IV	Fairly contaminated (Poor)	287
Sep – N	28	IV	Fairly contaminated (Poor)	341
Sep – R	4	V	Significantly contaminated (Very Poor)	67
Oct – Vt	126	I	Not contaminated (Excellent)	1302
Oct – S	143	I	Not contaminated (Excellent)	1377
Oct – Vr	114	I	Not contaminated (Excellent)	1172
Oct – N	116	I	Not contaminated (Excellent)	1235
Oct – R	10	V	Significantly contaminated (Very Poor)	151

Table 5 *(continued)*

BMWP' Month – Sites	Score	Class	Interpretation of Water quality	HS HBMWP
Nov – Vt	131	I	Not contaminated (Excellent)	1215
Nov – S	153	I	Not contaminated (Excellent)	1505
Nov – Vr	113	I	Not contaminated (Excellent)	1158
Nov – N	74	II	Evidence of contamination (Good)	735
Nov – R	4	V	Significantly Contaminated (Very Poor)	67
Dec – Vt	152	I	Not contaminated (Excellent)	1570
Dec – S	131	I	Not contaminated (Excellent)	1256
Dec – Vr	45	III	Contaminated (Moderate)	455
Dec – N	64	II	Evidence of contamination (Good)	641
Dec – R	5	V	Significantly Contaminated (Very Poor)	87
Jan – Vt	99	II	Evidence of contamination (Good)	1017
Jan – S	137	I	Not contaminated (Excellent)	1524
Jan – Vr	83	II	Evidence of contamination (Good)	789
Jan – N	48	III	Contaminated (Moderate)	481
Jan – R	7	V	Significantly Contaminated (Very Poor)	112
Feb – Vt	53	III	Contaminated (Moderate)	550
Feb – S	119	I	Not contaminated (Excellent)	1195
Feb – Vr	37	III	Contaminated (Moderate)	346
Feb – N	69	II	Evidence of contamination (Good)	697
Feb – R	5	V	Significantly Contaminated (Very Poor)	87

The vegetation structure of the riparian zone was of high complexity, while the predominant land-use consisted mainly of broadleaf and coniferous plantation. Only the site Vrontismeni was situated in an area where tilled land was the main land use. At the same site bank, reinforcement and embankments were the principal modifying factors and the basic feature of artificial influence was the presence of an irrigation network connected to the site. At sites Vrosina and Neraida quarrying activities were recorded, whereas at site Ragio the presence of a weir along the whole river width was considered of high modifying significance.

The Habitat Quality Score was ranged from 37 to 62 (Table 6), with site Ragio having the lowest score and site Neraida the highest one. Moreover, by comparing the HQA scores of each site with the HQA scores of sites of similar type, only sites Soulopoulo, Vrosina, and Neraida belonged to the top 20% of sites of similar type.

According to their Habitat Modification Scores (Table 6, see also Table 2), sites Soulopoulo and Vrosina were characterized as "semi-natural", site Ragio as "predominantly unmodified", site Neraida as "obviously modified", and site Vrontismeni as "obviously modified".

4 Discussion

Benthic invertebrates are considered to be good indicators of water quality last but not least because of their wide sensitivity variation towards contaminants and their relative immobility [6, 7, 28]. Point-pollution sources and diffuse loads of agricultural runoff affect macrobenthos communities and shift community composition from sensitive to tolerant taxa [6, 7]. In the case of Kalamas river, the recorded benthic communities and their abundance reflect human interferences. Plecoptera, which are the most sensitive to pollution insects [1, 7, 29, 30], presented low abundances at all sampling stations. Trichoptera and Ephemeroptera were found in high percentages at the upper land stations (sites Vrontismeni and Soulopoulo), probably due to the better water quality of these stations. The most tolerant taxa, such as the Dipteran families and Oligochaeta [31], were most abundant at the downstream stations reflecting the poorer water quality at these sites. Moreover, Chironomidae were

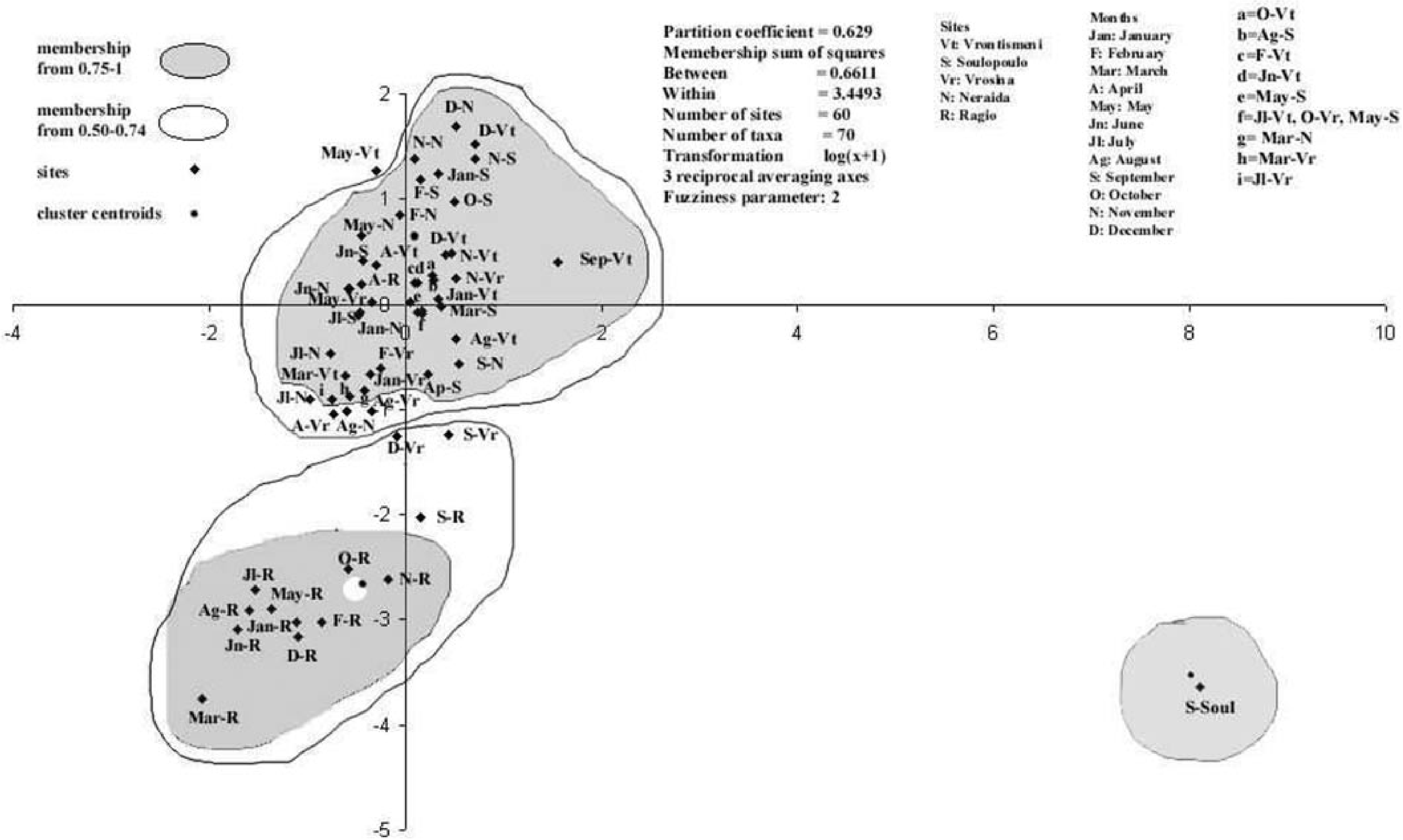

Fig. 4: Classification of Kalamas samples with FUZZY analysis according to the macroinvertebrate taxa found in them during sampling period March 2000 through February 2001. The criteria used for the analysis are given in the table above the diagram.

Klassifikation der Proben aus dem Kalamas River mittels FUZZY-Analyse bezogen auf die Makroinvertebraten im Untersuchungszeitraum März 2000 bis Februar 2001. Die für die Analyse verwendeten Kriterien sind in der Tabelle oberhalb des Diagramms dargestellt.

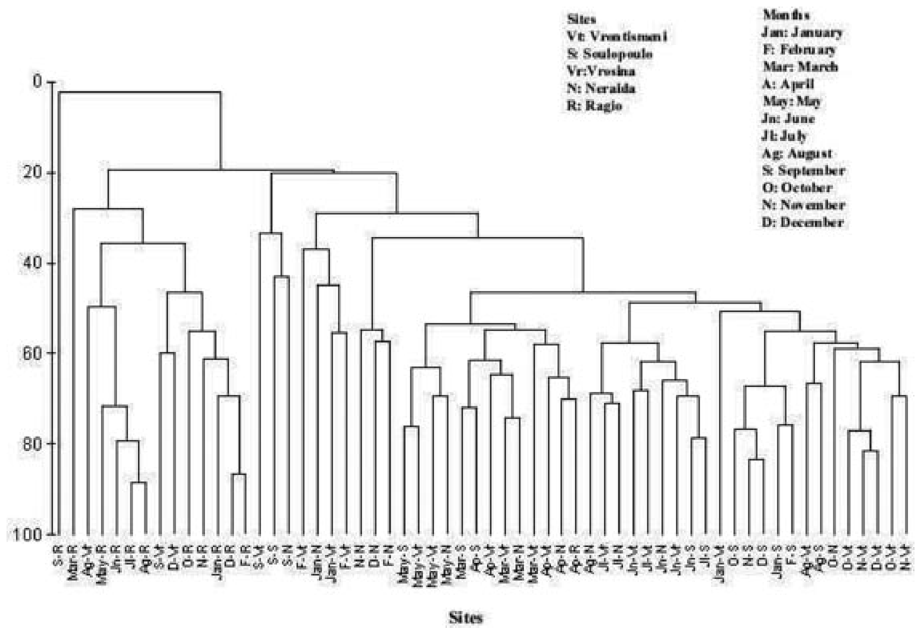

Fig. 5: Bray-Curtis similarity dendrogram of the Kalamas samples according to PRIMER analysis and to the macroinvertebrate taxa found in them during sampling period March 2000 through February 2001.

Ähnlichkeits-Dendrogramm nach Bray-Curtis der Proben aus dem Kalamas River bezogen auf die PRIMER-Analyse und die Taxa der Makroinvertebraten in den Proben vom März 2000 bis Februar 2001.

found to be the most abundant family at the most impacted site (Ragio), because of their tolerance to a broad spectrum of stressors [32]. Although Ragio was predominantly unmodified according to the Habitat Modification Score, it presented the lowest Habitat Qualification Score (Table 6).

The two biotic indices that were applied to the data proved to be effective in distinguishing the site of Ragio from the rest of the sites. During all year it showed the lowest abundances and biodiversity and was characterized as fairly or significantly contaminated site (Table 5), achieving the lowest Habitat Quality Assessment scores (Table 6).

Table 6: Habitat Quality Assessment (HQA) and Habitat Modification (HMS) scores at the 5 sites of Kalamas river.

Indices der Einschätzung der Habitatbeschaffenheit (HQA) und der Habitatmodifikation (HMS) an den fünf Probenahmestellen des Kalamas River.

Site	HQA	HMS
Vrontismeni	54	34
Soulopoulo	58	0
Vrosina	60	1
Neraida	62	11
Ragio	37	5

However, the biotic scores used were highest during the high temperature − low flow period. This finding is in disagreement with those of a previous study concerning Kalamas river using the macrophytic communities as bioindicators [17]. Other studies showed that in the Mediterranean aquatic systems, the water quality becomes worse during the extended dry period. According to Graca and Coibra [3] the seasonal changes in Mediterranean streams imply sequences of recolonizations, a process in which species may differ in their tolerance levels to environmental factors and presumably to pollution. Nevertheless, the identification and the application of the most appropriate indicators still remains a difficult task, because the sensitivity of taxa may vary according to the different phases of their life cycle [33], age [32], and geographical area [3]. The present study confirms the fact that further investigation is necessary to identify and develop good indicators applied to the particular conditions in the Mediterranean freshwater ecosystems.

FUZZY analysis differentiated Soulopoulo in September, because it was the only sample with high abundance of Limnephilidae (Trichoptera) (Fig. 4). It also differentiated Ragio samples from the rest, mainly because of the absence of the families of Elmidae (Coleoptera), Baetidae, Heptageniidae (Ephemeroptera), Hydropsychidae, and Glossosomatidae (Trichoptera) and their differences in the abundance of the families Ceratopogonidae and Chironomidae (Diptera) and the class of Oligochaeta. The families Heptageniidae and Glossosomatidae belong to the taxa that are characterized as sensitive to pollution [1, 11, 30] while various species of Chironomidae, Ceratopogonidae, and Oligochaeta are known to be tolerant to pollution and showed higher average abundances at Ragio samples. The only sample of Ragio that was grouped with the rest of the samples was that in April, which had significant numbers of Hydropsychidae, Glossosomatidae, Heptageniidae, and Baetidae. This grouping support the biotic indices, which characterized Ragio sites as sites with fairly or significantly contaminated waters (except of Ragio in April, Table 5).

The dendrogram created by PRIMER analysis was in accordance with the FUZZY clustering, since it differentiated Ragio samples from the rest (Fig. 5). However, PRIMER analysis isolated site Ragio in September, because of the only one Diptera tolerant family found there (Tabanidae).

The comparison of the habitat quality among the 5 sites cannot lead to conclusions unless sites of similar type are involved and reference conditions are defined [25, 26]. By performing a PCA analysis it was possible to determine the quality of the particular sites by comparing them with sites surveyed along Axios and Aoos rivers (N. Greece). As a result, sites Soulopoulo and Vrosina could be considered of a good habitat quality. Site Neraida, despite of its HQA score, cannot be considered equally good because of its modifications. Ragio and Vrontismeni were the poorest sites.

5 Conclusions

This approach was quite effective to characterize the quality of river water and the physical structure of the river habitats. The findings of the present study can be used as a "baseline" to assess the naturalness of the habitats and the modification level due to anthropogenic impacts. The classification of the river's sites, besides providing basic information on present environmental conditions, may also be used in the future for monitoring Kalamas river. This first attempt to apply the British RHS methodology in Hellas seemed to be satisfactory. however, it is necessary to determine more reference sites (benchmarks) for the calibration of the HQA scores.

Acknowledgements

This research had the financial support of the EU-LIFE projects (LIFE99ENV/G12/000557). We are grateful to the General Secretariat of Epirus region for its supporting.

References

[1] *Hynes, H. B. N.*: The Ecology of Running Waters. University Press, Liverpool, 1970.

[2] *Coimbra, C., Graca, M. A., Cortes, M. A.*: The effects of a basic effluent on macroinvertebrate community structure in a temporary Mediterranean river. Environ. Pollut. **94**, 301–307 (1996).

[3] *Graca, M. A. S., Coibra, C. N.*: The elaboration of indices to assess biological water quality. A case study. Water Res. **32**, 380–392 (1998).

[4] Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal L 327, 22 Dec 2000.

[5] *Metcalfe, L. J.*: Biological water quality assessment of running waters based on macroinvertebrate communities: history and present status. Europe Environ. Pollut. **60**, 101–139 (1989).

[6] *Karr, J. R., Chu, E. W.*: Restoring Life in Running Waters: Better Biological Monitoring. Island Press, Washington, D.C., 1999.

[7] *De Pauw, N., Vanhooren, G.*: Method for biological quality assessment of watercourses in Belgium. Hydrobiologia **100**, 153–168 (1983).

[8] *Eisler, R.*: Biological Science Report USGS/BRS/BRS, No 23, 1998.

[9] *Cajaraville, M. P., Abascal, I., Exteberria, M., Marigomez, I.*: Lysosomes as cellular markers of environmental pollution: time and dose-dependent responses of the digestive lysosomal system of muscles after petroleum hydrocarbon exposure. Environ. Toxicol. Water Qual. **10**, 1–8 (1995).

[10] *Castella, E., Bickerton, M., Armitage, P. D., Petts, G. E.*: The effects of water abstraction on invertebrate communities in U.K streams. Hydrobiologia **308**, 167–182 (1995).

[11] *Armitage, P. D., Moss, J. F., Wright, J. F., Furse, M. T.*: The performance of a new biological water quality score system based on macroinvertebrates over a wide range of unpolluted running water sites. Water Res. **17**, 333–347 (1983).

[12] *Maddock, I.*: The importance of physical habitat assessment for evaluating river health. Freshwater Biol. **41**, 373–391 (1999).

[13] *Arapis, G.*: Specific Environmental Impact Assessment Statement on Kalamas River. Perfecture of Thesprotia, Igoumenitsa, Greece, 1998 [in Greek].

[14] *Albanis, T., Pomonis, P., Sdoukos, A.*: Organophosphorous and carbamates pesticide residues in the aquatic system of Ioannina basin and Kalamas river (Greece). Chemosphere **15**, 1023–1034 (1986).

[15] *Stalikas, C. D., Pilidis, G. A., Karayiannis, M. I.*: Heavy metal concentrations in sediments of lake Ioannina and Kalamas river in north-western Greece. Fresenius Environ. Bull. **3**, 575–579 (1994).

[16] *Mane, F., Sakkas, V., Kalfakakou, V.*: Heavy metal pollution of Kalamas river. Hellenic Society for Biological Sciences, Proc. of the 23rd Panhellenic Conference, Chios, 2001, pp. 114.

[17] *Kagalou, I., Papastergiadou, E., Tsoumani, M.*: Monitoring of water quality of Kalamas river: a multivariate analysis. Freshwater Environ. Bull. (in press).

[18] APHA: Standard Methods for the Examination of Water and Wastewater. 16th Edition. American Public Health Association, New York, 1985.

[19] *Alba-Tercedor, J., Sanchez-Ortega, A.*: Un metodo rapido y simple para evaluar la calidad biologica de las aguas corrientes basado en el de Hellawell (1978). Limnetica **4**, 51–56 (1988).

[20] *Artemiadou, V., Lazaridou–Dimitriadou, M.*: Hellenic Evaluation Score and Interpretation Index of the quality of running waters in Northern and Central Greece, submitted.

[21] *Equihua, M.*: FUZZY clustering of ecological data. J. Ecol. **78**, 519–534 (1990).

[22] *Hill, M. O.*: DECORANA-A Fortran Program for Detrended Correspondence Analysis and Reciprocal Averaging. Ecology and Systematics. Cornell University, New York, 1979.

[23] *Field, J. G., Clark, K. R., Warwick, R. M.*: A practical strategy for analysing multispecies distribution patterns. Mar. Ecol. Prog. Ser. **8**, 37–52 (1982).

[24] *Raven, P. J., Fox, P., Everard, M., Holmes, N. T. H., Dawson, F. H.*: River habitat survey: a new system for classifying rivers according to their habitat quality. In: *Boon, P. J., Howell, D. L* (Eds.): Freshwater Quality: Defining the Indefinable? The Stationery Office, Edinburgh, 1997, pp. 215–234.

[25] *Raven, P. J., Holmes, N. T. H., Dawson, F. H., Everard, M.*: Quality assessment using river habitat survey data. Aquat. Conserv. Mar. Freshwater Ecosyst. **8**, 477–500 (1998).

[26] *Raven, P. J., Holmes, N. T. H., Dawson, F. H., Fox, P. J. A., Everard, M., Fozzard, I. R., Rouen, K. J.*: River Habitat Quality the Physical Character of Rivers and Streams in the UK and the Isle of Man. Environment Agency, Bristol 1998.

[27] *Mourkidis, G., Papadopoulou, E., Katsanos, A., Kakanis, P.*: Elemental analysis of water and sediments, Part 4: Aliakmon river, Greece. Chem. Ecol. **4**, 65–83 (1990).

[28] *Yousef, Y. A., Baker, D. Barbosa, F. A. R.*: Modeling and impact of metal accumulation in bottom sediments of wet ponds. Sci. Tot. Environ. **189/190**, 349–354 (2000).

[29] *Hawkes, H. A.*: Origin and development of the Biological Monitoring Working Party Score System. Water Res. **32**, 964–968 (1998).

[30] *Galdean, N., Callisto, M., Barbosa, F. A.*: Lotic ecosystems of Serra do Cipo, Southeast Brazil: water quality and a tentative classification based on the benthic macroinvertebrate community. Aquat. Ecosyst. Health Manage. **3**, 545–552 (2000).

[31] *Reece, P. F., Richardson, J. S.*: Benthic macroinvertebrates assemblages of coastal and continental streams and large rivers of Southwestern British Columbia, Canada. Hydrobiologia **439**, 77–89 (2000).

[32] USEPA: Macroinvertebrates Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters. EPA/600/4-90/030, 1990.

[33] *De Nicola, G. M., Migliore, L., Gambardella, C.*: Toxicity of mercury on different stages of the biological cycle of *Asellus aquaticus* and *Proasellus coxalis* Dollf (Crustacea, Isopoda). Bull. Soc. It. Biol. **62**, 321–328 (1986).

[Received: 1 July 2003; accepted: 26 February 2004]