Research Paper

Longitudinal impacts of anthropogenic pressures on benthic macroinvertebrate assemblages in a large transboundary Mediterranean river during the low flow period

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Changes in lotic benthic macroinvertebrate assemblages along the transboundary Axios-Vardar River (Greece – Former Yugoslavian Republic of Macedonia) were examined in order to identify major anthropogenic impacts correlated to the benthic community composition during the low flow season. Macrozoobenthos and water samples were collected from 21 sites during summer 2000 and beginning of autumn 2001. Parallel to sampling, the recording of the physical structure of the sites took place using the River Habitat Survey (RHS) method. The multivariate techniques of FUZZY and Canonical Correspondence Analysis (CCA), as well as the Hellenic biotic score (HES) and the habitat quality scores (HMS, HQA) were applied to the data. Total dissolved solids and total suspended solids were found to be the primary factors affecting the structure of the observed communities. Additionally, species composition responded to anthropogenic activities, e.g. untreated sewage effluents, industrial discharges, agricultural runoff, intense water abstraction and impoundment. As expected, macrozoobenthos community composition shifted from sensitive to tolerant taxa where human impacts were most evident.

Keywords: Biomonitoring / ecological integrity / river habitat survey / Axios-Vardar / Water Framework Directive

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1 Introduction

Studies on large Mediterranean rivers are scarce with the exception of river systems in France and the Iberian Peninsula [1, 2]. Every river change almost entirely as one travels from the headwaters downstream but the hydrology of Mediterranean rivers exhibits vast changes due to prolonged arid summers. With regard to water quality assessment, periods with low river discharge are to be preferred for monitoring because pollution is then more pronounced [3, 4]. Invertebrate communities change in response to changes in physicochemical factors [5] and available habitats. The biotic structure and water quality of streams and rivers reflect an integration of the physical, chemical and anthropogenic processes occurring in a catchment area, leading to the concept of ecological integrity. Ecological integrity is defined as the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms, having a composition and diversity comparable to that of the natural habitats in the region [6]. Moreover, this concept implies an equally integrated ecological assessment [7], using a variety of parameters to detect the different types of disturbances. Human induced hydrological changes, physical disturbances (habitat alteration, urban land use) and point and non-point sources of pollution (chemical contamination, surface runoff, intensive agriculture) are examples of processes responsible for a broad-scale deterioration of lotic ecosystems [7].

Benthic macroinvertebrates are considered the most appropriate biological indicators of water quality in many EU countries [8]. Their advantages are widely discussed by many authors [8–12]. They are also one of the

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prerequisite parameters (biological quality components) for monitoring under the Water Framework Directive (WFD) 2000/60/EC [13]. At the same time the evaluation of physical structure and habitat quality of rivers are used to anticipate the hydromorphological reporting monitoring requirements of the WFD [14, 15].In the present study, the longitudinal pattern of the benthic macroinvertebrate assemblages along the Axios-Vardar River was investigated in order to define the factors that influence their composition during the low flow period and to determine biotic changes due to anthropogenic pressures. This river was chosen because it is a major transboundary river in the South Balkans and of great economic and ecological importance for Greece and the Former Yugoslavian Republic of Macedonia (FYROM). Additionally, it is the very first time that a common integrated water quality assessment survey has been carried out in both countries. Biomonitoring assessment of Axios-Vardar was previously carried out only in the Greek part of the river [16–18]. No available similar data exist for the river part in FYROM.

2 Study area description

Axios-Vardar is one of the largest transboundary rivers in the Balkan Peninsula. Its springs are situated in the Scardos and Sar mountains, in the FYROM. It discharges into the Thermaikos Gulf in Northern Greece. Its total length is approximately 388 km, of which 77.6% belongs to FYROM. The river flows with a mean channel slope of 1.72 ‰, with 2.12 ‰ and 0.35 ‰ in FYROM [19] and Greece [20] respectively. The catchment area is 23 747 km^2 with 86.5% in FYROM and 13.5% in Greece (http://www.inweb.gr/workshops/sub_basins/10_Axios.html, 2004). Major tributaries are the Treska, Lepenic, Pcinja, Bregalnica, Crna in FYROM, and Arzan-Ayak, Kotza, Gorgopis, Vardarovasi in Greece. The annual mean discharge at the delta is 137 $m^3 s^{-1}$ with extreme variations (minimum flow in late summer-autumn and maximum in late winter-spring). Due to the irrigation dam Fragma Elli (26 km upstream from the delta), which remains closed from May to September, discharge falls dramatically during the dry season [20].

Intense agriculture takes place in the river catchment area. River water is used primarily for irrigation as well as for industrial and domestic use. Legal and illegal water abstraction for irrigation causes significant fluctuations to the river's flow regime. Agricultural run-off leads to high amounts of pesticides and fertilizers detected in the river water [21]. Organochlorine compounds have also been found, in some cases even higher than the EU limit values [22]. The river receives untreated effluents from many urban settlements and industrial discharges in both countries [23]. Additionally, significant sand extraction and quarrying activities are carried out at several points.Although the lower part of the river is strongly modified – it has been channelized and diverted since 1934 – the river's delta along with the vicinal delta still comprises an internationally important wetland belonging to the Ramsar Convention. It is also protected as a Special Protected Area (SPA), a Special Area of Conservation (SAC) and a "Natura 2000" site by the EU Directive 92/43/EEC [24]. Fish fauna is comprised of 26 species, one of which is endemic [*Pachychilon macedonicum* (Steindachner)] [25].

3 Materials and methods

Benthos and environmental variables were collected at 9 sampling sites in the FYROM part of the river (Vardar) during the beginning of autumn 2001 and at 12 sites along the Greek part of the river (Axios) during summer 2000 (Fig. 1). Three sites were situated on tributaries (Breganica (AR10), Gorgopis (AG21) and Vardarovasi (AB7)). All sites were chosen in a stratified random way, but not in the same pace for both countries. Habitat evaluation features were recorded on a 500 m reach upstream of each sampling location.

3.1 Physicochemical parameters

Dissolved oxygen concentration (DO), saturation of dissolved oxygen (DO%), temperature, pH, conductivity and total dissolved solids (TDS) were measured *in situ* with portable polymeters (YSI 55, Metler Toledo) and the appropriate probes. Surface water samples were taken in polypropylene bottles and stored at 4°C for further laboratory analysis of PO_4^{3-}-P, NO_3^--N, NO_2^--N, NH_4^+-N and total suspended solids (TSS) concentrations according to APHA [26]. Additionally, two water samples were collected in 300 mL BOD bottles and sealed in the dark at 20°C. BOD_5 was determined as the difference in dissolved oxygen measured at the time of collection and again five days later with an oxygen meter (YSI 55).

3.2 Hydromorphological parameters, habitat features and habitat evaluation

Substrate was estimated visually as percentage occurrence of each particle category using the Wentworth scale [27]. Channel width was measured at the same transects, with a tape-meter and where not possible with a rangefinder (Bushnell 400). Channel depth and water velocity were measured at transect equidistant points

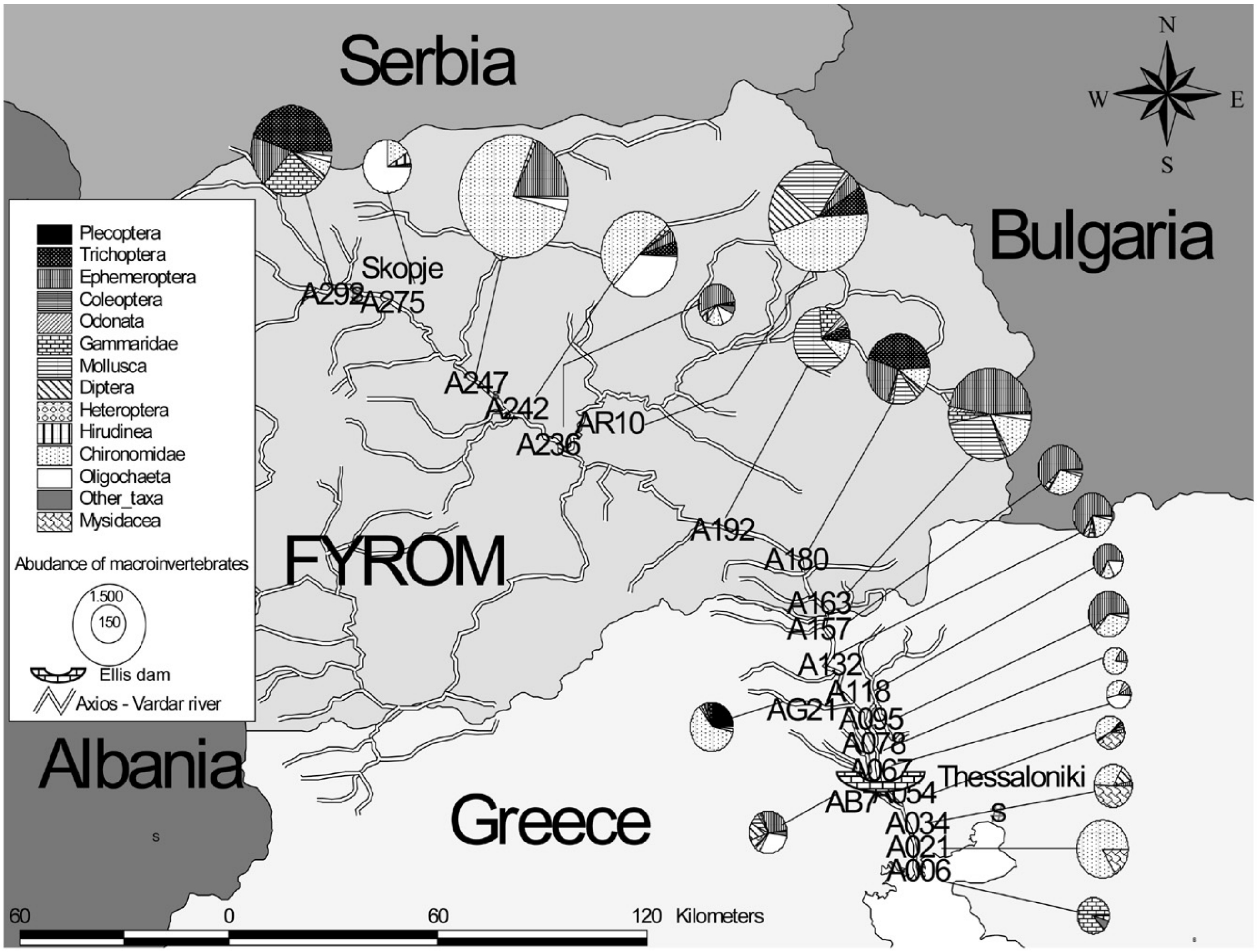


Figure 1. Catchment of Axios-Vardar River and its tributaries. The sampling sites are coded so that the first letter refers to the river; if present a second letter to the tributary and the 3-digit number refers to the division of the river into 500 m sections from the river mouth or the tributary junction. The main cities are given with a dot. Pies represent the percentage composition of the benthic fauna while their size is relative to the total macroinvertebrate abundance at each site.

when possible using a flow meter (Swoffer 2100) so as to calculate discharge [28]. At each site, instream and bank vegetation cover was estimated visually as percentage occurrence.

Habitat evaluation was based on field assessment protocols of the River Habitat Survey (RHS) [29, 30] completed in the same period with the macrozoobenthos sampling. RHS assesses the structural variability of the river habitats. It lies on the basis that the river biota respond to a set of interlinked physical subsystems – the ‚habitat templet' of Southwood [31] – by allocating different organisms to occupy different subsystems. It includes observations of substrate, flow, erosional and depositional features in the channel, morphological and vegetation structure on the banks, and land use in the adjacent river corridor along a standard 500 m sample unit [32–34]. Collected data were computerized using the RHS 3.2 database [35]. Habitat Quality Assessment (HQA) and Habitat Modification Scores (HMS) were calculated with HQA scoring system version 1.2 and HMS rules version 1.1 [33] in order to assess the habitat structure quality and the extent of human alteration of each site. Principal Component Analysis (PCA) was applied to HQA scores [36] in order to compare the habitat quality of the studied sites with sites of similar type already surveyed in rivers of the South Balkans.

3.3 Benthic macroinvertebrates and biotic scores

Benthic macroinvertebrates were sampled in each site with the 3-minute kick-sweep method [37, 38], using a standard pond net (surface 575 cm^2, mesh size 900 µm, depth 27.5 cm). During the three-minute period, all exist-

Table 1. Greek Habitat Richness Matrix. A site can be classified as rich, if at least one checked type of habitat belongs to the diagonal striped cells; otherwise it is classified as poor.

If present	Macrophyte bed	Natural substrate			Artificial substrate	Slough	Woody snag
		Coarse *	Mixture **	Fine ***			
Riffle							
Channel margin							
Island margin							
Main channel							
Run							
Channel margin							
Island margin							
Main channel							
Pool							
Channel margin							
Island margin							
Main channel							

* Coarse: Substrate composition >70% of boulders and/or cobbles and/or pebbles
** Mixture: Variant substrate composition that cannot be classified as coarse or fine
*** Fine: Substrate composition >70% of gravel and/or sand and/or silt

ing instream habitat types at each site (macrophyte beds, woody snags, bars, natural or artificial substrates at riffles, runs and pools) were sampled, according to Methods for Collecting Benthic Macroinvertebrate Samples by USGS [39]. Samples were sorted in the laboratory using a 0.5 mm mesh sieve and identified down to the lowest possible taxonomic level.

The biotic Hellenic Evaluation Score (HES) [40] was applied on macroinvertebrate data. It originated from the Iberian Biological Monitoring Working Party (B.M.W.P.') [41] but has received an additional number of families, a reassessment of all family scores so as to meet the specific conditions of Greek rivers and it also takes into account the relative abundance of the different taxa. With the addition of the Greek Habitat Richness Matrix (GHRM) (Table 1), HES is standardized against the rich or poor types of habitat variations. GHRM is a modification from the US Environmental Protection Agency protocol for macroinvertebrate sampling [39].

3.4 Statistical analyses

FUZZY clustering technique was used to classify the sites, based on the similarity of their benthic macroinvertebrate communities from the taxa pool data [42]. While in most clustering methods in case of an object lying between two clusters, it must be assigned to one of them, in FUZZY clustering, each observation is given fractional membership in multiple clusters [43]. This method has been applied consistently to invertebrate site assemblage data and enables the most parsimonious groups to be derived from data that consist of irregular gradients [44]. The participation values of the sites were used for the classification. This method was preferred due to the fact that it does not assume the existence of discrete benthic populations along the various stretches of a river system.

Ordination was carried out by Canonical Correspondence Analysis (CCA) [45]. CCA is a direct ordination technique, which means that the environmental variables are directly related to the species composition at the sites. The ordination axes in CCA are chosen as linear combinations of the environmental variables [46]. Monte Carlo permutation test was used to test the significance of every variable contributing to the model ($p > 0.05$). The samples labelled as river sites were projected in the ordination diagrams, which included the first and second ordination axes. CCA was carried out using Canoco for Windows 4.02 [47].

All macroinvertebrate data were transformed to $\log(x+1)$ before the statistical analyses to approach assumed conditions of normality and homocedasticity [48].

4 Results

4.1 Physicochemical parameters

The results of the physicochemical characteristics of the river water are presented in Table 2 and the correspond-

Table 2. Values of physicochemical parameters at Axios-Vardar sites (summer 2000 – autumn 2001). Values exceeding the EU suggested or permitted levels for water intended for human consumption (98/83/EC) are indicated with bold characters.

Site	DO %	DO mg L^{-1}	pH	Temperature, °C	Conductivity, mS cm^{-1}	TDS mg L^{-1}	TSS mg L^{-1}	PO_4^{3-}-P µg L^{-1}	NH_4^+-N µg L^{-1}	NO_2^--N µg L^{-1}	NO_3^--N µg L^{-1}	Flow m s^{-1}	Discharge m^3 s^{-1}
A292	99.2	9.29	8.1	18.8	306	153	48	990	144.3	8.06	2 933.5	5.23	–
A275	56.2	5.11	7.8	19.9	364	204	24	122.6	**899.8**	47.66	2 009.2	0.95	–
A247	129	10.97	8.2	23.3	408	206	46	87.8	387.1	86.38	3 600.6	1.81	–
A242	129	10.84	8.4	24.3	448	225	44	131.8	468.1	86.38	3.9	3.86	–
A236	115	9.34	7.1	25.8	436	218	1	584.1	400.6	80.56	4 033.4	4.43	–
A192	117	9.65	8.5	24.7	553	283	0.8	1 474.8	76.8	7.77	3 105.0	2.65	–
A180	125	10.03	8.7	26.2	700	322	14	1 198.8	171.3	10.97	3 663.3	2.15	–
A163	175	13.73	9	27.8	634	321	24	1 363.5	265.7	12.43	3 245.1	5.9	–
A157	84.5	7.7	7.7	19.9	456	233	63.7	899.6	42.2	4.88	1 591.9	0.59	24.83
A132	100	8.68	8.4	23.2	474	242	80.1	905.5	18.2	3.81	1 481.6	–	–
A118	98.2	8.3	8.4	23.7	522	258	125	1 055.6	159.9	65.55	1 521.2	–	–
A095	79.1	6.73	8	23.1	550	278	125.2	871.2	32.6	10.42	905.2	0.39	–
A078	88.2	7.44	7.9	23.5	565	284	125.6	754.5	30	7.84	874.0	0.3	–
A067	68.5	5.65	8	23.3	528	260	38.8	972.7	412.1	15.38	751.2	0.01	–
A054	111	8.84	8.3	26.6	735	369	91.2	1 052.9	52.3	22.38	755.3	0.05	–
A034	108	8.41	8.5	27.6	762	381	118.5	1 328.5	57.5	11.93	570.1	0.16	0.92
A021	124	9.6	8.7	28.3	829	415	97.3	1 018.6	219.6	23.94	684.6	0.3	–
A006	135	10.6	8.8	28.1	**4 860**	2 430	63.4	649.9	5.2	10.42	430.7	0.07	–
AB7	77.7	6.65	7.6	23.7	938	468	28.4	886.9	27.6	7.71	382.8	0	0.05
AG21	87	7.82	7.3	19.8	632	317	91	42.8	110	0.23	245.5	0.05	0.01
AR10	137	11.02	8.8	27.2	1 018	506	32	194.5	292.7	8.35	824.4	1.51	0.88

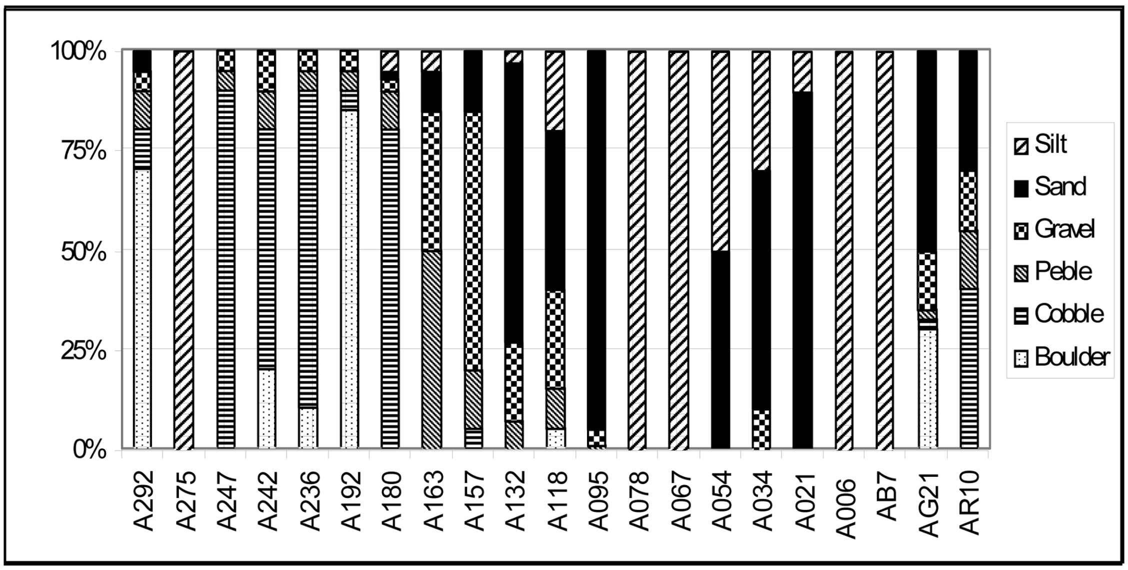


Figure 2. Percentage composition of substrate at sampling sites on Axios-Vardar River (summer 2000 – autumn 2001).

ing substrate composition in Figure 2. The discharge at the Greek border (A157) was 24.83 m^3 s^{-1}, but dropped dramatically after Ellis dam to 0.92 m^3 s^{-1} (A034). Thus, water temperature, dissolved oxygen concentration and pH values differentiated dramatically at sites downstream of the irrigation dam. Conductivity and total dissolved solids were high at all sites. Especially at site A006 (2.5 km before the river mouth) the highest values of conductivity (4860 mS cm^{-1}) and total dissolved solids (2430 mg L^{-1}) were recorded, due to the intrusion of seawater. Total suspended solids concentrations varied from 0.8 mg L^{-1} (A192) to 125.6 mg L^{-1} (A078). Ammonium concentration was found to exceed the EU levels at site A275 downstream of Skopje.

4.2 Habitat evaluation

Table 3 shows the output from the RHS analysis. The impact of human activities on the physical structure of the river was reflected in the HMS values. Most of the surveyed sites were characterized as "semi-natural" or "predominantly unmodified", whereas six as "significantly" or "severely modified". Upstream sites in FYROM had higher HQA scores than lowland ones in Greece. Major human interference (HMS) was mainly due to channel

Table 3. Habitat Modification Scores and Class (HMS), Habitat Quality Assessment (HQA) scores, abundances and taxa of benthic macroinvertebrates, Hellenic Evaluation Score (HES) and Average (AHES) biotic scores applied to Axios-Vardar sites (summer 2000 – autumn 2001).

	River habitat quality			Biotic score				
Site	HMS Score	HMS Class	HQA Score	Abundance	Taxa	HES	AHES	Water quality
A292	84	Severely modified	44	1 747	18	1 072	51.05	Moderate
A275	23	Significantly modified	42	498	3	173	34.60	Bad
A247	3	Predominantly unmodified	62	3 285	11	574	44.15	Bad
A242	20	Obviously modified	54	1 530	15	722	42.47	Bad
A236	0	Semi-natural	49	239	8	396	44.00	Moderate
A192	6	Predominantly unmodified	48	772	21	1 175	51.09	Moderate
A180	5	Predominantly unmodified	42	988	16	978	57.53	Good
A163	2	Semi-natural	46	1 832	19	1 102	52.48	Moderate
A157	8	Predominantly unmodified	52	406	13	523	40.23	Bad
A132	0	Semi-natural	47	297	14	678	48.43	Moderate
A118	8	Predominantly unmodified	41	121	6	168	33.60	Bad
A095	1	Semi-natural	49	319	9	362	40.22	Moderate
A078	13	Obviously modified	33	49	4	144	36.00	Bad
A067	10	Obviously modified	46	53	6	220	36.67	Moderate
A054	22	Significantly modified	41	113	7	330	47.14	Bad
A021	4	Predominantly unmodified	37	612	8	330	41.25	Moderate
A006	1	Semi-natural	29	158	3	116	38.67	Bad
AB7	45	Severely modified	16	264	20	837	52.31	Bad
AG21	8	Predominantly unmodified	50	385	13	686	36.12	Moderate
AR10	0	Semi-natural	60	2 665	14	644	49.54	Moderate

modification and extensive embankments, quarrying and agricultural activities. Sites A292 and AB7 proved to be severely modified whereas site AR10, at the Bregalnica tributary, was found to have the most diverse river habitat.

4.3 Benthic macroinvertebrates and biotic scores

16 604 individuals were sampled and 66 benthic macroinvertebrate taxa identified during this study. Invertebrate abundance was much higher at the sites sampled in FYROM than at sites in Greece (Fig. 1). The lowest abundances were recorder at sites A078 and A067 with 49 and 53 individuals respectively. Site A247 had the highest abundance. Sites A06 and A292 had the lowest taxa diversity, whereas A192 had the highest (Table 3).

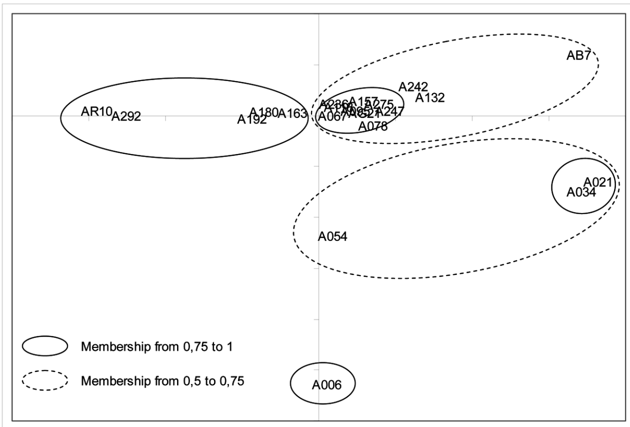
Diptera larvae, predominantly families Simuliidae and Chironomidae, were the most abundant taxon in almost all sites (Fig. 1). The abundance of Chironomidae varied from 1.7 to 83.5% among the sites. Ephemeroptera constituted the second most abundant taxon especially at upstream sites in FYROM, with Caenidae and Baetidae being the most abundant families. Trichoptera were also mainly found at upstream sites and in tributaries, with Hydropsychidae being the dominant family. Gastropoda were collected primarily at sites A192, A180, A163 and AR10. At lowland sites they were almost absent (Fig. 1).

Regarding the sensitive high-scoring taxa, only one Plecopteran family (Nemouridae) were collected at site AG21 in Gorgopis tributary. Sensitive trichopteran families, Psychomyidae and Sericostomatidae, were found at sites A292, A192, A180 and A163. Concerning the tolerant low-scoring taxa, Oligochaeta and Hirudinea had the highest occurrence at site A275 below the city of Skopje. Benthic composition of site A006 was totally different than the rest of the stations due to its proximity to the river mouth and the presence of sea water. The remaining taxa (Decapoda, Neuroptera, Isopoda, Polychaeta, Acari, Platelminthes) never constituted more than 8.2% of the macroinvertebrate fauna at any site.

Biotic scores and the classification of the sites according to the Hellenic Evaluation Score (HES) are represented in Table 3. Almost all sites had either bad or moderate water quality according to HES interpretation. The lowest values of HES were recorded below the city of Skopje (A275), at site A118 and at the last site (A006) before the river mouth. On the contrary, sites A192, A180, A163 and AR10 had a high HES score, as well as site A292 upstream of the city of Skopje. Good water quality was found only at site A180.

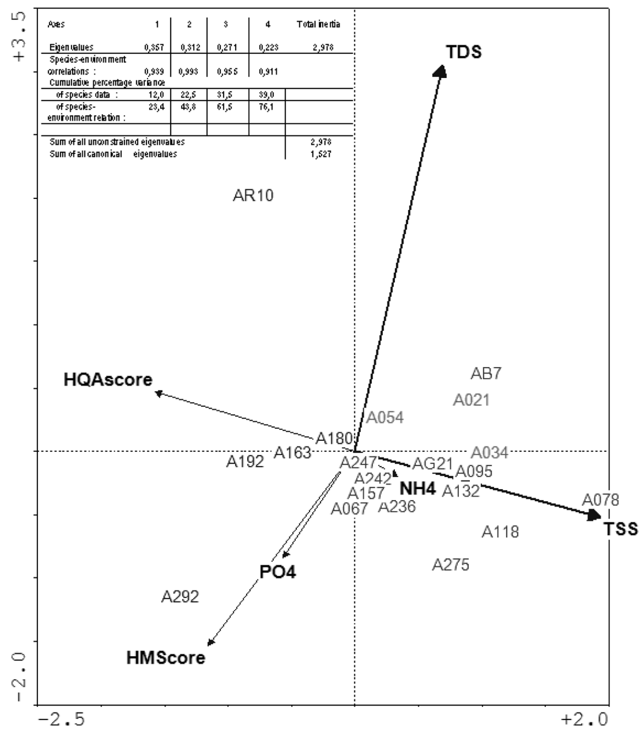
4.4 Statistical analyses

FUZZY analysis grouped the sampling sites into four clusters (Fig. 3). It clearly separated site A006 from the rest of the sites as a cluster of its own (D), revealing its peculiar benthic composition and physicochemical character due to the intrusion of seawater. For this reason it was excluded from the rest of the analyses to avoid misclassifications of the rest of the samples. Cluster A comprised the high biotic HES scoring sites. The composition of the benthic macroinvertebrate community was so different at sites A054, A034 and A021 below the dam, as to justify their separation from all others in a different cluster (C).



Partition coefficient: 0.75366851178612
Number of individuals (rows): 21
Number of features (columns): 66
Number of clusters: 4
data transformed into: Log(X+1)
Analysis using 2 reciprocal averaging axes
Method: fuzzy CMeans
alpha factor: 0
Fuzziness parameter: 2.1
Norm: Diagonal
Convergence criterion: 0.00000000001

Figure 3. Canonical Correspondence Analysis (CCA) diagram with environmental variables and 20 sites on the Axios-Vardar River, summer 2000 – autumn 2001. Site A006 was excluded from the analysis due to its outstanding different benthic composition. CCA parameters are given in the table below. Best interset correlations of environmental variables with axes are in bold.



Interset correlations of environmental variables with axes				
	Axis 1	Axis 2	Axis 3	Axis 4
Eigenvalues	0.1017	0.1842	0.1258	0.0889
TDS	0.1865	**0.8937**	0.0192	0.0793
TSS	**0.5188**	-0.1501	-0.0089	0.6390
PO_4^{3-}	-0.1528	-0.2463	-0.3600	-0.1489
NH_4^+	0.0892	-0.0574	-0.0944	0.2178
HMSscore	-0.3094	-0.4488	0.7111	0.1945
HQAscore	-0.4231	0.1370	-0.3320	0.1059

Figure 4. Classification of the Axios-Vardar River sites with the FUZZY method based on the invertebrate fauna. Site A006 was not included in the analysis due to its outstanding different species composition. The convergence criterion and other parameters are indicated below.

The rest of the sites formed cluster B consisting of low water quality sites.

The eigenvalues of the first two axes of CCA accounted for 70% of the variance (Fig. 4). TSS was the variable best correlated with the first axis, whereas the second axis was best correlated with TDS. All high HES score sites were plotted on the negative side of axis 1, having a positive relation to the HQA score (Fig. 4). All sites on the Greek part of the river were placed on the positive side of axis 1 together with sites downstream of major cities in FYROM. These sites had high values of TSS values, fine substrate material and high nutrient load, while the majority of them had pollution tolerant families.

5 Discussion

River management (e.g. impoundments, water abstraction, channel modifications), point sources of pollution and diffuse loads of agricultural runoff, all affect macrozoobenthos assemblages. In the case of the Axios-Vardar River, biotic scores and statistical analyses helped to assign distinct catchment and anthropogenic impacts to the benthic macroinvertebrate assemblages along the river gradient during the low flow period, when pollution and hydrological impacts are most evident. At sites where human pressures were present, taxa tolerant to pollution, such as Chironomidae, Oligochaeta and Hirudinea increased in abundance, while non-tolerant ones decreased (e.g. some Ephemeroptera families). Sensitive Plecoptera taxa were absent from all sites except from site AG21 located on a tributary. Their absence is related to low discharge (abstraction) and high water temperatures. This change in the benthic composition has been already observed in other river systems, where anthropogenic impacts were most evident [49–52].

More specifically, regarding the FYROM sampling sites, FUZZY analysis separated sites A292, A192, A180, and A163 from the rest of the stations (Fig. 3). Although, they were distanced from point sources of pollution nutrient levels were high. Nevertheless, these sites showed the highest HES scores and taxa richness. Consequently, the benthic composition at these sites comprised of the high scoring taxa Aphelocheiridae, Athericidae and high densities of Baetidae and Caenidae. Prosobranch Gastropoda (Hydrobiidae, Bithynidae, Neritidae) and Elminthidae were found only at sites A192, A180 and A163, presumably due to their requirements for well-oxygenated water conditions [53]. At the same sites, RHS revealed a diverse instream environment and absence of major human impacts. Although RHS results do not relate directly physical habitat features with benthic communities [54], they may indirectly imply the influence of riverine processes on water quality helping to predict benthos functional organization [55]. Indeed, Canonical Correspondence Analysis (CCA) showed that especially sites A192, A180 and A163 were correlated positively to the HQA scores. The almost unregulated state and increased channel of the river along these sites allows the free development of geomorphological features, such as riffles, pools, erosion and depositional features, which favours the diversity of ecological processes in the river channel. Sites with low biotic score families and poor taxa biodiversity were clustered together by FUZZY analysis. Their fauna consisted mainly of the burrowing Chironomidae and Oligochaeta, which are tolerant to suspended solids and silt substrates [56]. Site A275 located downstream of Skopje, the capital of FYROM, was characterized by high pollution levels due to untreated sewage effluents; only Chironomidae, Glossiphoniidae and Oligochaeta were collected there. Sites A247 and A242 were still under the influence of the severe pollution load originated from Skopje. Consequently, macroinvertebrate assemblages were dominated by Chironomidae and Oligochaeta (80%). However, at downstream sites (A192, A180), the confluence of Bregalnica and Babuna tributaries combined with the improved river habitat structure in the Vardar gorge (manifested by the HQA score), ameliorated the water quality, due to dilution and self-purification processes.

In the Greek part of the river, agricultural run-off, abattoir wastes and sewage effluents in Axioupoli were the major anthropogenic activities influencing the biota [18, 57]. The toxic effects of pesticides originating from agricultural run-off and the presence of pollution tolerant taxa in the downstream part of a river are well documented elsewhere [52] and are also of importance in this case. From site A132 to A067, macroinvertebrate abundances and biotic scores were lower in comparison to the upstream sites of FYROM. Chironomidae abundance was reduced, while other taxa such as Odonata were totally absent. The most important anthropogenic influence though, was the impoundment located at site A067. Such impoundments are well known to be associated with alteration of temperature regimes and dissolved oxygen concentrations [58], discharge variation [59], reduction of sediment load [60], variation in food type and availability [61], and even habitat change [62]. Indeed, the immediate downstream sites (A054, A034, A021) showed higher values of dissolved oxygen concentrations, pH and water temperature, while flow and total suspended solids levels were reduced. It seems that silt covers the sand and pebble substrate as long as the dam's portal remains open, whereas, when the portal closes, the water free of TSS – due to sedimentation in the reservoir – washes away the silt and the coarser substrate is uncovered. Coarser substrate and water free of total suspended solids resulted in more complex macroinvertebrate communities. Thus, the abundance of the benthic macroinvertebrates increased in comparison to the upstream sites and Chironomidae became the predominant family [58, 62], while Ephemeroptera decreased, as was also found by Pardo *et al.* [61]. Not surprisingly, these sites were clustered together in the FUZZY analysis (Fig. 3).

The most severe impact of the impoundment was evident at the river mouth at site A006. The increased use of water during summer for irrigation purposes leads to minimum flows and to the consequent intrusion of seawater at lowland sites, turning freshwater to brackish in Mediterranean rivers [63]. Site A006, situated close to the river mouth (3 km upstream) was clearly separated by FUZZY analysis (Fig. 3) due to the domination of its benthic composition by Myscidacea, Polychaeta and Gammaridae. Moreover, the extremely high conductivity and TDS values reveal intrusion of seawater. Skoulikidis *et al.* [64] have also reported extreme values for conductivity along the lowland part of Axios-Vardar, due to the dramatic abstraction of the river water during the low flow period.

CCA analysis indicated that the patterns of macroinvertebrate assemblages were primarily associated with total dissolved solids and total suspended solids. These results generally agree with the ones found by Kampa *et al.* [18] and Lazaridou-Dimitriadou [23] in the same river, correlating total suspended solids with tolerant families. Total suspended solids have also been related to observed macrofauna patterns in other rivers of North Greece [17, 51, 65–67] and most probably constitute one of the determining mechanisms of the distribution of macrozoobenthos in South Balkan river systems.

Habitat Quality Scores (HQA) reflect the variety in habitat characteristics recorded by RHS in a site [29]. It is expected that the greater the scores are, the more taxa will be anticipated. Nevertheless, in the present study (Fig. 5a) ($R^2 = 0.239$, $p = 0.039$) no such pattern was clearly demonstrated. However, HQA had a slight positive effect

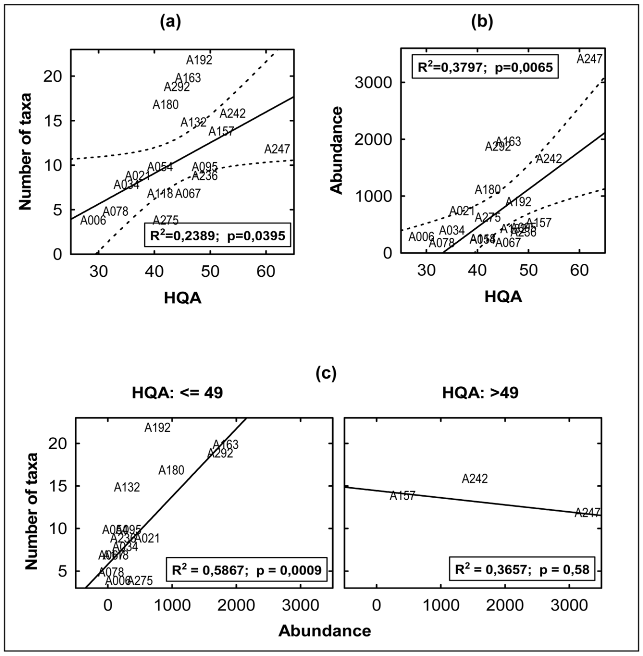


Figure 5. Linear regression of Habitat Quality Assessment (HQA) scores, abundances and number of taxa found at each sample along the Axios-Vardar River, summer 2000 – autumn 2001. (a) HQA scores over number of taxa found at each sample ($n = 18$). (b) HQA scores over abundances found at each sample ($n = 18$). (c) Number of taxa over abundances found at each sample, for different ranges of HQA scores.

on species abundances (Fig. 5b) ($R^2 = 0.340$, $p = 0.006$). When samples are grouped according to their HQA scores, the number of taxa is positive correlated to the abundance for low HQA, but not for high HQA scores (Fig. 5c). This is related to the fact that sites that represent a high variety of habitats are not necessarily natural ones. Site A247 had both the highest HQA score and the largest abundance, but it was dominated by the tolerant family of Chironomidae. The high HQA score was rather the effect of the various recorded macrophytes, which at the same time were favoured by the high nutrient levels caused by organic pollution from Skopje. Therefore, the high HQA score in the case of A247 reflects in fact a disturbance happened several kilometres upstream, and although it offers available habitat features (macrophytes) for colonisation [68], it is not related to the macroinvertebrate community found.

Despite the fact that extreme seasonal variation is an inherent component in determining macrozoobenthos assemblages in Mediterranean river systems, in the case of Axios-Vardar River such assemblages face parallely a plethora of anthropogenic pressures. Stress becomes intense especially during the low flow season with considerable negative consequences on the composition of such communities.

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