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Assessing the Ecological Integrity of a Major Transboundary Mediterranean River Based on Environmental Habitat Variables and Benthic Macroinvertebrates (Aoos-Vjose River, Greece-Albania)

key words: biomonitoring, multivariate analysis, reference conditions, river habitat survey

Abstract

Ecological integrity has become a primary objective in monitoring programs of surface waters according to the European Water Framework Directive. For this reason we propose a scheme for assessing the ecological integrity of a major transboundary river, the Aoos-Vjose (Greece-Albania), by analysing the effects of physicochemical, hydromorphological and habitat structure variables on benthic macroinvertebrates. Benthos and water samples were obtained from 17 sites, during high and low flow season. Physical habitat structure was determined using the River Habitat Survey method. In all but one of the surveyed habitats no anthropogenic change was evident. Macrobenthos assemblages were mainly influenced by seasonality and river section, whereas the water quality index was negatively correlated to habitat modification. Consequently, a large part of the river is considered of high ecological integrity and as such it may be used as baseline information for the management of other major rivers in the eastern Mediterranean basin.

1. Introduction

Many approaches have been proposed for the evaluation of the quality of running waters. In the past, monitoring was based on physical and chemical characteristics of the water bodies. In recent years, assessment methods are starting to include other instream, riparian and catchment level characteristics (Bis *et al.*, 2000). This approach reflects the concept of ecological integrity, the evaluation of which has become a primary objective of monitoring programs of surface waters (Barbour *et al.*, 2000). Ecological integrity of streams and rivers can be defined as a minimal deviation from natural reference conditions (Bunn and Davies, 2000). To determine the state of ecological integrity of a given water body, one requires the recording of a variety of parameters in order to detect different types of disturbances (*e.g.*, physical habitat impairment, hydrological fluctuations, physicochemical degradation, biotic alterations). River courses of high integrity are expected to be governed solely by natural factors, such as biogeography, seasonality, or spatial characteristics.

The ecological integrity approach has been incorporated in the EU 2000/60 Water Framework Directive (WFD). According to the WFD, EU member states are expected to establish monitoring networks in order to determine the "ecological status" of their river courses by using biological, physicochemical and hydromorphological elements (Irmer, 2000; European Commission, 2003). Ecological status must be assessed by the Ecological Quality Ratio

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which is done by ranking the similarity distance between the current state of a river body and its reference values for biological elements, in comparison to the expected physicochemical and hydromorphological elements for the given biological conditions (European Commission, 2003). Reference values are derived from specific localities on a river which are minimally impaired and representative of the expected ecological integrity in similar river localities (Economou, 2002).

Whereas the WFD obliges member states to achieve at least a good quality status before 2015, no Balkan country has developed, to our knowledge, a concrete method for identifying the ecological river status yet. In this study, we evaluate the ecological integrity of Aoos-Vjose River by assessing the effects of physicochemical, hydromorphological parameters and habitat structure have on benthic macroinvertebrates with the use of multivariate statistics. In cases of unknown stress types if only one organism group can be investigated, then benthic macroinvertebrates should be considered since they respond to most stressor types in all river types (Hering *et al.*, 2006). Our results indicate that localities along Aoos-Vjose river can be used for the identification of reference, potential reference and/or best attainable ecological potential conditions (Chaves *et al.*, 2006) for the management of other large river systems in the eastern Mediterranean basin.

2. Materials and Methods

2.1. Study Area Description

Aoos-Vjose is a large transboundary river in the South Balkan Peninsula, according to the criteria for large rivers in system A, in Annex II of the WFD (European Commission, 2000) with a river basin area of 6710 km^2. It is the only river that originates in Greece and descends to a neighbouring country (Albania) with its estuary in the Adriatic Sea (Fig. 1). The hydrological regime of the river reflects the Mediterranean climate of its basin with characteristic discharge extremes in late summer-autumn and in late winter-spring. The underlying geology consists mainly of calcareous dolomites, flysch, ophiolites, serpentines, psammitic and alluvial deposits. Aoos-Vjose has a high mean annual sediment transport of approximately $4.85 \cdot 10^6$ tons (Lazaridou *et al.*, 2002).

In the Greek part, forestry, cattle breeding and isolated aquaculture units are present but limited due to the roughness of the terrain. In the Albanian part, agriculture and cattle breeding are the most important activities. The river receives untreated effluents from 5 urban settlements (Konitsa, Permet, Argirokastro, Tepelen, Mamalje, Selenica), small-scale industrial discharges and in the lower parts by-products of petroleum extraction. Minor abstraction activities also take place for irrigation purposes and for water supply to rural settlements.

During spring and autumn 2001, we collected benthos and environmental variables at 17 sites, in a stratified random sampling scheme along the river (Fig. 1; Table 1). Major tributaries were included in the monitoring and the stratum was predetermined at 1 site every 20 km. At each site, samples were taken during the high flow season (Spring 2001) (hereafter denoted with H), and the low flow season (Autumn 2001) (hereafter denoted with L). Only site VA17 (headwaters) was inaccessible to sample during Spring 2001. Habitat features were recorded only once, in Autumn 2001, on a 500 m reach upstream of each sampling point.

2.2. Validation of Reference Sites

We applied the validation criteria found in Chaves *et al*. (2006), adapted for Greek rivers (Table 2), for the identification of reference sites (REF). With the exception of the tributaries we did not consider the first step which consists of site selection and inspection, because although none of the sites failed in more than 3 of the criteria relative to this step, one of the main tributaries affecting all sites downstream from V543 has a large dam in its springs. Dams are considered as exclusive criteria for reference sites according to Chaves *et al*. (2006) at small rivers. However, the impact of this dam is expected to be minimized, after a considerable amount of discharge is entering the Aoos-Vjose in the Alban-

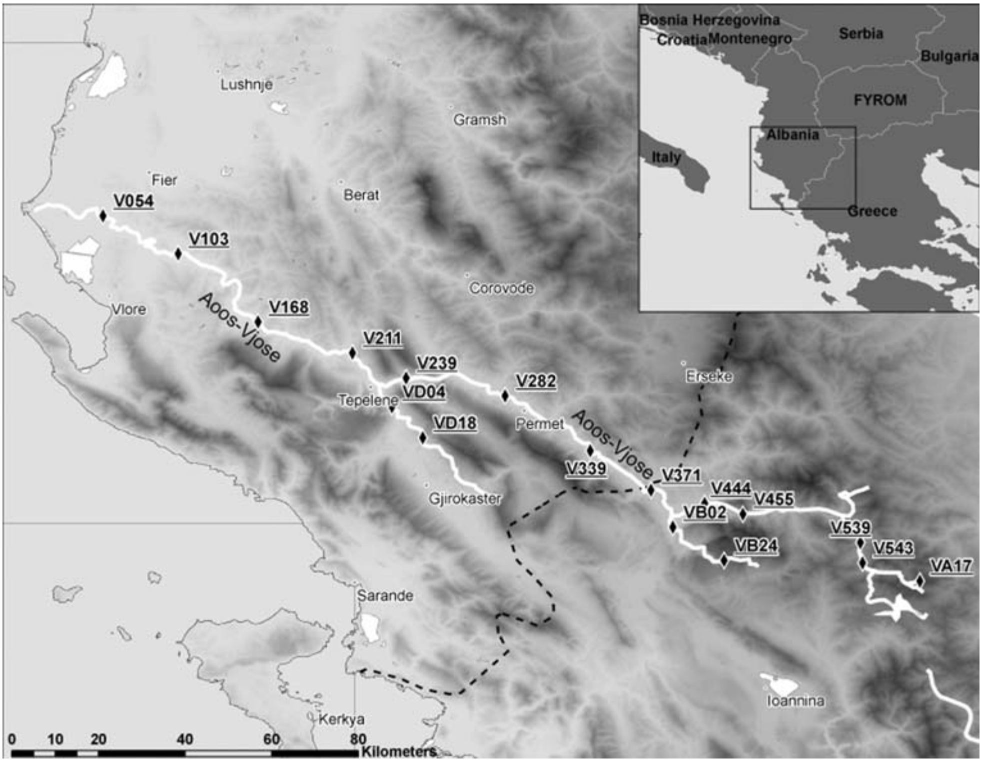

Figure 1. Location of the 17 sampling sites at the Aoos-Vjose river and its basic tributaries (Spring-Autumn 2001). The sampling sites are coded so that the first letter refers to the river; the second letter to the tributary (if present) and the 3-digit number refers to the division of the river into 500 m sections from the river mouth or the tributary junction.

ian part, since the ratio of the enclosed basin to the unregulated one gets less than 0.2. Thus, we proceeded at the main channel sites only to the validation step in order to find out the availability for comparisons of these sites as Potential Reference sites (PREF) and Best Attainable Ecological Potential (BAEP) (Chaves *et al*., 2006).

2.3. Physicochemical Variables

We measured temperature, pH, conductivity, total dissolved solids (TDS), dissolved oxygen concentration (DO) and oxygen saturation (DO%) *in situ* using portable meters (YSI 55, Metler Toledo) with the appropriate probes.

Surface water samples were taken in polypropylene bottles and stored at 4 °C. Additionally, two water samples were collected in 300 ml BOD bottles and sealed in the dark at 20 °C. We determined BOD_5 as the difference in dissolved oxygen measured at the time of collection and five days later with an oxygen meter (YSI 55, Metler Toledo).

Table 1. Location of the 17 sampling sites at Aoos-Vjose river and its basic tributaries (spring-autumn 2001).

Site	River	Location	Altitude (m)	Distance from Source* (km)	Watershed (km^2)	Longtitude	Latitude
V054	Aoos-Vjose	(Mifol)	15	248.5	6690	19.49 E	40.61 N
V103	Aoos-Vjose	(Upstream Selenica)	65	224	5570	19.65 E	40.54 N
V168	Aoos-Vjose	(Kalivaci)	82	191.5	5420	19.82 E	40.39 N
V211	Aoos-Vjose	(Downstream Tepelene)	115	170	3920	19.01 E	40.32 N
VDO4	Drinos	(Lekli)	134	66.5	1300	20.05 E	40.26 N
VD18	Drinos	(Shtepesi)	140	59.5	1005	20.08 E	40.23 N
V239	Aoos-Vjose	(Dragot)	170	156	2350	20.11 E	40.30 N
V282	Aoos-Vjose	(Downstream Permet)	200	134.5	1960	20.30 E	40.26 N
V339	Aoos-Vjose	(Dracova)	290	106	1730	20.49 E	40.13 N
V371	Aoos-Vjose	(Burazani)	340	90	1170	20.62 E	40.06 N
VBO2	Voidomatis	(Upstream river confluence)	360	22.5	370	20.65 E	40.00 N
VB24	Voidomatis	(Springs)	460	11.5	275	20.69 E	39.95 N
V444	Aoos-Vjose	(Downstream Konitsa)	420	58	703	20.72 E	40.03 N
V455	Aoos-Vjose	(Aoos Gorge)	500	52.5	630	20.77 E	40.25 N
V539	Aoos-Vjose	(Downstream Vovusa)	980	15	232	21.04 E	39.95 N
V543	Aoos-Vjose	(Papa rema)	1000	14	176	21.05 E	39.91 N
VA17	Arkoudorema	(Saliatouras)	1520	2	>0.5	21.16 E	39.87 N

* While several springs contribute to the discharge, the source is considered the most distant one (Raven *et al.*, 1998).

2.4. Hydromorphological Variables, Habitat Features and Habitat Evaluation

We estimated the substrate composition visually as the percentage occurrence of each particle category using the Wentworth scale (Wentworth, 1922). Channel depth and water velocity were measured at equidistant points along a transect in every site using a flow-depth meter (Swoffer 2100). Discharge was calculated from channel dimensions and flow measurements (Horne and Goldman, 1983). At each site, we estimated the macrophyte river bed cover visually as percentage occurrence.

Habitat evaluation was based on the River Habitat Survey (RHS) (Raven *et al.*, 1997; UK Environment Agency, 1997). It involves the systematic recording of features associated with the physical structure of the river's watercourse along a standard 500 m sample unit (Raven *et al.*, 1998a, 1998b). It includes observations of substrate, flow, erosional and depositional features in the channel, morphological, vegetation structure on the banks, land use in the adjacent river corridor (Fox *et al.*, 1998; Raven *et al.*, 2002), and human modifications which interrupt the longitudinal or lateral continuum with the adjacent ecosystem. We processed our RHS data with the RHS 3.2 database (UK Environment Agency, 2000). Habitat Quality Assessment (HQA) and Habitat Modification Scores (HMS) were calculated to assess the habitat structure quality and the extent of human alteration at each site. As a standard procedure of the RHS system, Principal Component Analysis (PCA) was applied to the HQA scores (Jeffers, 1998) in order to compare the habitat quality of the studied sites with sites of a similar type already surveyed in rivers of the South Balkans (Chatzinikolaou *et al.*, 2006).

Table 2. Criteria used in the validation process (CHAVES *et al.*, 2006) adapted for Greek rivers in order to estimate the disturbance level in the Aoos-Vjose River sites.

Criteria	Abbreviation	Description	Bibliography
Hellenic Assessment System	HESII	>3	ARTEMIADOU and LAZARIDOU, 2005
Habitat Quality Assessment score	HQA	>35	
Water physicochemical parameters		Natural concentrations of physicochemical parameters measured in water	
Ammonia	NH_4	<0.0610 mg/l at basins <900 km^2	SKOULIKIDIS *et al.*, 2006
		<0.3900 mg/l at basins >900 km^2	(BRUNEL *et al.*, 1997 in SKOULIKIDIS *et al.*, 2006)
Nitrates	NO_3	<0.6100 mg/l at basins <900 km^2	SKOULIKIDIS *et al.*, 2006
		<5.6000 mg/l at basins >900 km^2	(BRUNEL *et al.*, 1997 in SKOULIKIDIS *et al.*, 2006)
Nitrites	NO_2	<0.0081 mg/l at basins <900 km^2	SKOULIKIDIS *et al.*, 2006
		<0.0500 mg/l at basins >900 km^2	(BONADA *et al.*, 2002 in CHAVES *et al.*, 2006)
Phosphates	PO_4	<0.1060 mg/l at basins <900 km^2	SKOULIKIDIS *et al.*, 2006
		<0.1630 mg/l at basins >900 km^2	(BRUNEL *et al.*, 1997 in SKOULIKIDIS *et al.*, 2006)

2.5. Benthic Macroinvertebrates and Biotic Scores and Indices

We sampled benthic macroinvertebrates at each site with the 3-minute kick-sweep method (ARMITAGE *et al.*, 1983; ARMITAGE and HOGGER, 1994), using a standard pond net (surface 575 cm^2, mesh size 900 μm, depth 27.5 cm). During the three-minute method, we collected macrobenthos from all existing instream habitat types at each site (macrophyte beds, woody snags, bars, natural or artificial substrate at riffles, runs and pools) (CHATZINIKOLAOU *et al.*, 2006) and preserved them in 4% formaldehyde. In the laboratory, animals were sorted and identified down to family level.

We applied the Greek biotic evaluation scheme (ARTEMIADOU and LAZARIDOU, 2005) on the macroinvertebrate data. This scheme includes the biotic Hellenic Evaluation Score (HBMWP), the average Hellenic evaluation Score Per Taxon (HASPT) and the Hellenic Evaluation Score interpretation index (HES). The HES provides information about the sensitivity of every macroinvertebrate family to pollution. To standardize the HES against rich or poor types of habitat variations, we used the Greek Habitat Richness Matrix (GHRM) (CHATZINIKOLAOU *et al.*, 2006), modified from the USEPA protocol for macroinvertebrate sampling (CUFFNEY *et al.*, 1993).

2.6. Statistical Analyses

We applied the Mann-Whitney test to compare means of the physicochemical parameters between the two flow periods. A non parametrical Spearman correlation was used to detect whether the habitat and the biotic scores are correlated.

We applied the FUZZY clustering technique (EQUIHUA, 1990) to obtain both ordination and classification of the sampling sites based on the similarity of their benthic macroinvertebrate communities. We preferred this method because it does not assume the existence of discrete benthic populations along the various stretches of a river system. Prior to the analyses, we transformed all macroinvertebrate data to $\log(x + 1)$ to approach conditions of normality and homocedasticity (SOKAL and ROHLF, 1987). Coupled to FUZZY we used SIMPER analysis performed by Primer 5.1.2 for Windows to explain which macroinvertebrate families contribute to the similarity or dissimilarity between the clusters produced by FUZZY (CLARKE and WARWICK, 1994).

Canonical Correspondence Analysis (CCA) was performed in order to detect covariance between environmental variables and abundances of taxa (TER BRAAK, 1988). Correlated variables were excluded with the use of the inflation factor and the Monte Carlo permutations test. The latter test also provided the significance of every environmental variable contributing to the model ($P < 0.05$). CCA was carried out on the log-transformed macroinvertebrate data using Canoco for Windows 4.02 and the produced CCA graph was produced by CanoDraw 3.1 and CanoPost 1.0.

3. Results

Upper reaches of Aoos-Vjose were characterized mainly by coarse substrate material (boulders, cobbles, pebbles), reaching in most of the cases 60% of the total cover (Fig. 2). Typically, fine particle materials were observed mainly in lowland sites, where sand and silt composition increased towards the river mouth. Conductivity, total dissolved solids and total hardness were significantly higher ($P < 0.05$) during the low flow period (Table 3). Only total suspended solids were lower during the low flow period ($P = 0.033$). Ortho-phosphate and ammonium concentrations were found to be high at site VB02 during autumn (low flow). Values of total suspended solids, total hardness, pH and alkalinity are indicative for medium-hard, alkaline water with a high buffering capacity.

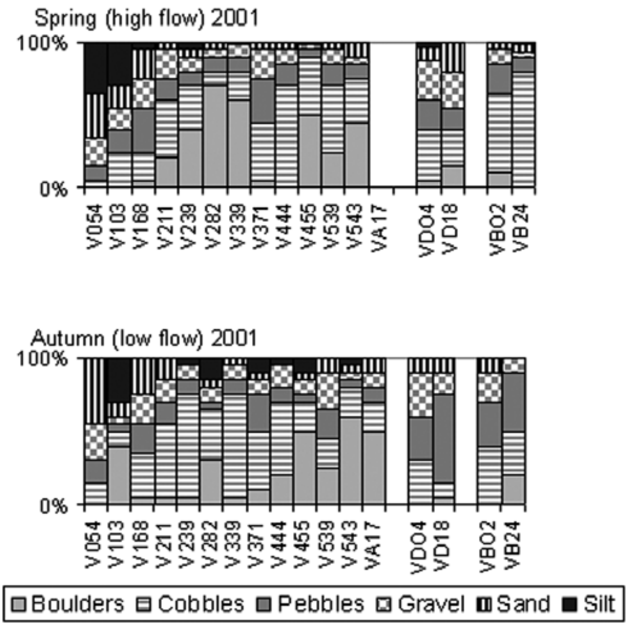

Figure 2. Percentage composition of channel substrate at the sampling sites at the Aoos-Vjose river during Spring and Autumn 2001.

Table 3. Results of physicochemical variables and discharge at 17 sites along Aoos-Vjose in Spring and Autumn 2001. Parameters underlined represent significant differences between the two seasons ($P < 0.05$, Mann-Whitney). Values exceeding the validation criteria in Table 2 are bold.

Site	DO (%)	DO (mg/l)	BOD_5 (mg/l)	pH	Temp. (°C)	Cond. (µS/cm)	Alkal.	TDS (mg/l)	TSS (mg/l)	Total hardness	PO_4-P (mg/l)	NO_2-N (mg/l)	NO_3-N (mg/l)	NH_4-N (mg/l)	Discharge m^3/s
	Spring														
V054	97.8	10.04	1.62	8.1	16.0	502	166.0	255	95.0	215.0	0.0270	0.0075	1.20	0.040	182.0
V103	97.6	10.80	2.49	8.2	15.1	436	160.0	226	65.0	212.5	0.0120	0.0060	1.25	0.035	159.0
V168	97.2	10.35	0.16	8.1	13.2	410	116.0	212	45.0	135.0	0.0240	0.0040	1.20	0.000	152.0
V211	99.8	10.54	3.34	8.1	13.0	451	159.0	231	0.0	192.5	0.0100	0.0035	1.85	0.000	144.0
VD04	97.7	10.90	1.43	8.1	14.1	475	168.0	249	0.0	212.5	0.0280	0.0078	1.40	0.036	42.5
VD18	95.9	9.97	2.32	7.9	13.9	501	171.0	258	0.0	217.5	0.0070	0.0030	1.15	0.000	0.0
V239	97.3	10.48	2.56	8.2	12.6	463	240.0	240	15.0	195.0	0.0120	0.0050	1.50	**1.250**	94.3
V282	102.4	11.20	2.48	8.3	11.6	469	170.0	242	20.0	210.0	0.0280	0.0078	1.40	0.036	85.0
V339	96.9	10.86	2.22	8.4	10.3	416	161.0	211	45.0	210.0	0.0350	0.0120	1.20	0.075	61.0
V371	97.2	11.01	2.99	8.1	9.6	339	125.9	171	9.0	285.7	0.0143	0.0041	0.32	0.008	49.0
VB02	89.8	10.20	1.93	7.8	9.4	276	59.8	138	273.0	125.0	0.0276	0.0023	0.06	0.078	106.1
VB24	96.2	11.14	2.04	7.9	8.9	230	104.6	117	50.0	214.3	0.0152	0.0020	0.09	0.015	47.4
V444	97.1	11.50	2.90	8.3	8.1	265	122.7	131	84.0	232.1	0.0124	0.0006	0.08	0.000	8.0
V455	94.3	11.26	2.81	8.3	7.9	264	121.6	133	83.0	214.3	0.0143	0.0006	0.08	0.000	35.0
V539	92.3	11.25	1.27	8.1	7.1	190	61.4	98	95.0	125.0	0.0086	0.0000	0.06	0.000	6.1
V543	93.6	11.49	2.44	8.1	6.8	180	54.4	86	171.0	89.3	0.0190	0.0012	0.06	0.005	29.0
VA17	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Autumn														
V054	94.1	9.39	1.20	7.8	15.4	608	160.0	304	0.0	265.0	0.0000	0.0045	1.12	0.000	35.0
V103	112.5	10.77	2.00	8.1	17.3	630	150.0	322	15.0	290.0	0.0040	0.0080	0.05	0.000	28.0
V168	104.5	10.50	1.80	7.8	15.1	602	150.0	281	0.0	205.0	0.0035	0.0050	1.25	0.000	25.0
V211	113.8	11.62	2.20	8	14.4	641	150.0	359	6.0	275.0	0.0040	0.0090	1.20	0.000	19.0
VD04	103.5	10.56	0.95	7.9	14.6	613	180.0	352	0.0	290.0	0.0080	0.0040	1.44	0.035	4.0
VD18	109.0	11.05	1.55	7.9	14.7	660	170.0	326	0.0	275.0	0.0048	0.0020	1.00	0.035	2.7
V239	87.3	9.13	1.30	7.8	13.2	848	170.0	441	0.0	295.0	0.0040	0.0060	0.85	0.000	13.4
V282	130.1	12.75	1.70	8.5	16.3	502	150.0	298	0.0	245.0	0.0035	0.0120	1.03	0.000	13.2
V339	167.5	14.80	0.85	8.7	15.4	521	145.0	264	5.0	260.0	0.0110	0.0150	1.40	0.050	10.0
V371	120.0	12.60	3.74	8.4	14.3	483	72.6	271	77.7	285.7	0.0375	0.0034	1.16	0.182	6.3
VB02	75.3	7.98	5.17	7.1	12.5	425	69.4	217	38.9	250.0	**0.1258**	0.0055	0.56	0.702	2.0
VB24	80.1	9.16	2.95	7.9	9.2	272	53.4	148	52.4	142.9	0.0158	0.0010	0.55	0.012	12.0
V444	93.3	10.33	2.64	8	10.9	349	96.0	173	56.6	267.9	0.0192	0.0010	0.12	0.032	1.5
V455	93.9	10.06	4.35	8	12.3	337	104.6	183	51.7	178.6	0.0208	0.0011	0.15	0.052	4.6
V539	107.7	10.79	3.23	8.9	15.2	299	101.4	150	24.3	142.9	0.0192	0.0011	0.09	0.061	0.0
V543	93.4	9.76	3.35	8.8	13.3	247	93.9	129	22.1	321.4	0.0192	0.0010	0.06	0.032	3.4
VA17	82.9	9.69	3.20	8.1	8.7	183	74.7	92	7.1	214.3	0.0208	0.0014	0.20	0.052	0.1

Table 4. Abundance, number of taxa of the 17 sampling sites during Spring and Autumn 2001 in Aoos-Vjose river. Water quality indices involve the Hellenic Evaluation Score (HES), Average Hellenic Evaluation Score per taxon (AHES) and the Hellenic Evaluation Score Interpretation Index (HESII). Habitat quality assessment scores (HQA) and habitat modifications scores (HMS) calculated during the low-flow period (Autumn 2001).

	Spring (high flow)						Autumn (low flow)										
Site	Abundance	Taxa	HBMWP	HASPT	HES	Water Quality	Abundance	Taxa	HBMWP	HASPT	HES	Water Quality	Major type of modification	HQA Score	HMS Score	HMS Class	HMS Interpretation
V054	40	9	333	41.63	2.5	Moderate	384	15	771	55.07	4	Good	–	45	2	1	Semi-natural
V103	218	11	492	49.20	3.5	Good	139	19	935	51.94	4	Good	Embankment, water abstraction	48	6	2	Predominantly unmodified
V168	868	18	972	54.00	3	Moderate	872	18	1137	63.17	3.5	Good	Unfinished bridge	55	4	2	Predominantly unmodified
V211	545	20	1211	60.55	3.5	Good	6689	21	1401	66.71	4.5	High	–	37	2	1	Semi-natural
VD04	1770	17	1050	61.76	3.5	Good	3462	22	1497	68.05	5	High	Bank reinforcement	54	4	2	Predominantly unmodified
VD18	4934	16	1036	64.75	4	Good	2795	29	1745	62.32	4.5	High	–	54	0	1	Semi-natural
V239	653	16	958	59.88	4.5	High	7104	23	1417	61.61	4	Good	Unfinished bridge	46	4	2	Predominantly unmodified
V282	2969	22	1415	64.32	4	Good	1868	20	1313	65.65	4	Good	Gravel extraction	42	3	2	Predominantly unmodified
V339	4283	25	1525	61.00	4	Good	5190	29	1905	65.69	5	High	–	39	0	1	Semi-natural
V371	2537	36	2110	60.29	4.5	High	7332	25	1636	65.44	5	High	–	46	0	1	Semi-natural
VB02	5649	36	2143	63.03	4.5	High	4963	27	1421	54.65	4	Good	–	58	0	1	Semi-natural
VB24	2112	25	1589	69.09	5	High	3406	23	1611	67.13	5	High	–	55	0	0	Pristine
V444	1146	23	1606	69.83	5	High	1595	20	1394	69.70	5	High	Gravel extraction, Channel resection, Embankment	37	24	4	Significantly modified
V455	120	16	1050	65.63	4.5	High	434	23	1526	66.35	5	High	Bank reinforcement	54	8	2	Predominantly unmodified
V539	1214	29	1923	68.68	5	High	877	29	1808	64.57	4.5	High	–	55	0	1	Semi-natural
V543	293	26	1619	64.76	5	High	285	23	1510	65.65	4.5	High	–	58	0	0	Pristine
VA17	Not accessible						480	26	1710	68.40	5	High	–	45	0	0	Pristine

Site V444 was the only one characterized as "severely modified", according to the River Habitat Survey. V444 had the highest HMS value, due to modifications at banks and the river channel caused by quarrying activities. Regarding the habitat quality, VB02 had the richest score (HQA) whereas sites V211 and V444 the poorest (Table 4).

We identified in total 77226 benthic macroinvertebrates which belonged to 75 different taxa. Abundances were found to be higher in the middle part of the river during both seasons (Table 4, Figs. 3 and 4). Ephemeroptera appeared to be the most abundant order in almost all sites during the high flow season (Fig. 2). Predominant families were those of Heptageniidae, Ephemerellidae, Caenidae and Baetidae. Trichoptera constituted the second most abundant order, and during the low flow period their family, Hydropsychidae, became dominant. Regarding the high-scoring taxa, Plecoptera were recorded at all upstream sites (Fig. 3) with Leuctridae being the most abundant family during low flow. Concerning the low-scoring taxa, Diptera were present in all sites with Chironomidae as their most abundant family. Sites V371, V239 and V211 during low flow conditions presented the highest abundances, while V054 during high flow presented the lowest.

Almost all samples had high biotic scores and were classified as of good quality according to the HES (Table 4). The only exceptions were samples collected at sites V054 and V168 during high flow conditions. Autumn samples at lowland sites achieved higher biotic scores than during spring.

FUZZY analysis grouped the samples according to their fauna into five clusters (Fig. 5) with the use of 2 reciprocal axes and a resulting partition coefficient of 60.6%. Cluster A comprised basically the low flow season samples. Cluster C consisted of upland high flow samples. The rest of the high flow samples were grouped in cluster E. They were all situated in the lowland Albanian part of the river. Cluster D was comprised only of the Voidomatis's tributary site VB24 (both high and low flow samples). The composition of the macroinvertebrate community has been found so different in sample V054H, situated close to the river mouth, as to justify its separation from the rest of the sites as a different cluster (B) (Fig. 5). Samples of Voidomatis's site VB02 were not included in any cluster.

FUZZY multivariate analysis provided clusters of the samples based on seasonality, river tributary and longitudinal gradient (Table 5). Families of Elminthidae and Leuctridae seemed to be the taxa that contributed the most to the formation of the low flow samples cluster A. Cluster D differed from the rest due to the presence of Sericostomatidae and Thremmatidae. The presence of Ephemerellidae distinguished cluster E from the rest. Families of Leuctridae, Elminthidae and Hydropsychidae were responsible for the differences between clusters of high and low flow.

According to the forward selection of the Monte Carlo permutation test only 10 out of the 31 environmental variables were due to contribute to the CCA plot (Fig. 6). The first two axes of the ordination explained the 37.1% of the variance of the species-environmental variables relation. The most important variables of those correlated to axis I were alkalinity, river tributary, total suspended solids and distance from source. High flow samples were placed on the positive side of axis II, whereas low flow samples were placed on the negative side. Axis II was correlated to season and discharge. From the positive towards the negative side of axis I, samples were distributed from the upland to the lowland sites and according to the tributary they belonged to.

The validation procedure assessed VA17 and VB24 tributaries sites as REF, while VD18, VD04 and VB02 sites may still be considered as PREF. Failure in one reference nutrient concentration was considered acceptable for PREF and BAEP. The rest sites 13 sites of Aoos-Vjose are selectable as PREF or BAEP.

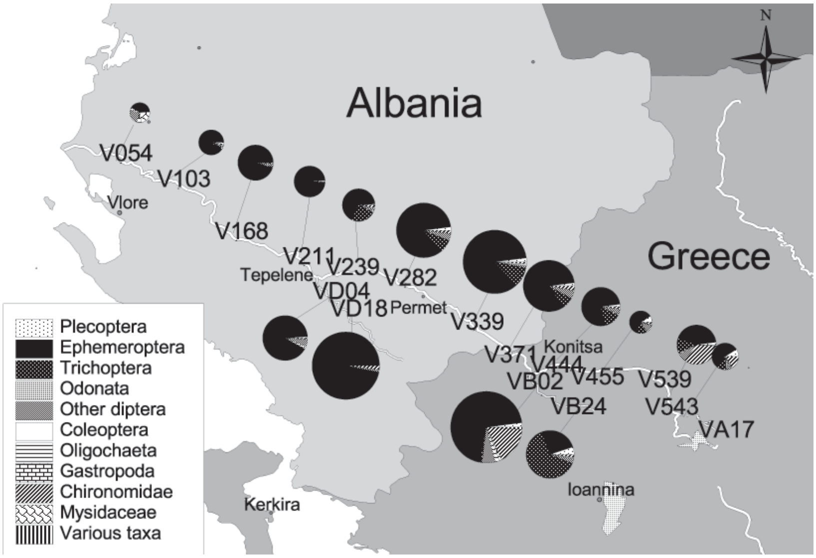

Figure 3. Sites at the catchment of Aoos-Vjose river and its tributaries. Pies represent the percentage composition of the benthic fauna at each site during Spring 2001 (high flow period), while their size is proportional to the abundance found.

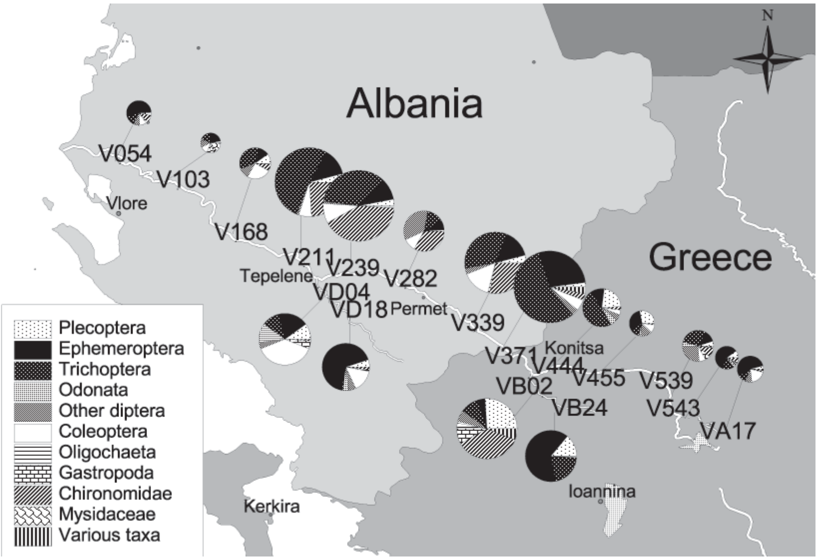

Figure 4. Sites at the catchment of Aoos-Vjose river and its tributaries. Pies represent the percentage composition of the benthic fauna at each site during Autumn 2001 (low flow period), while their size is proportional to the abundance found.

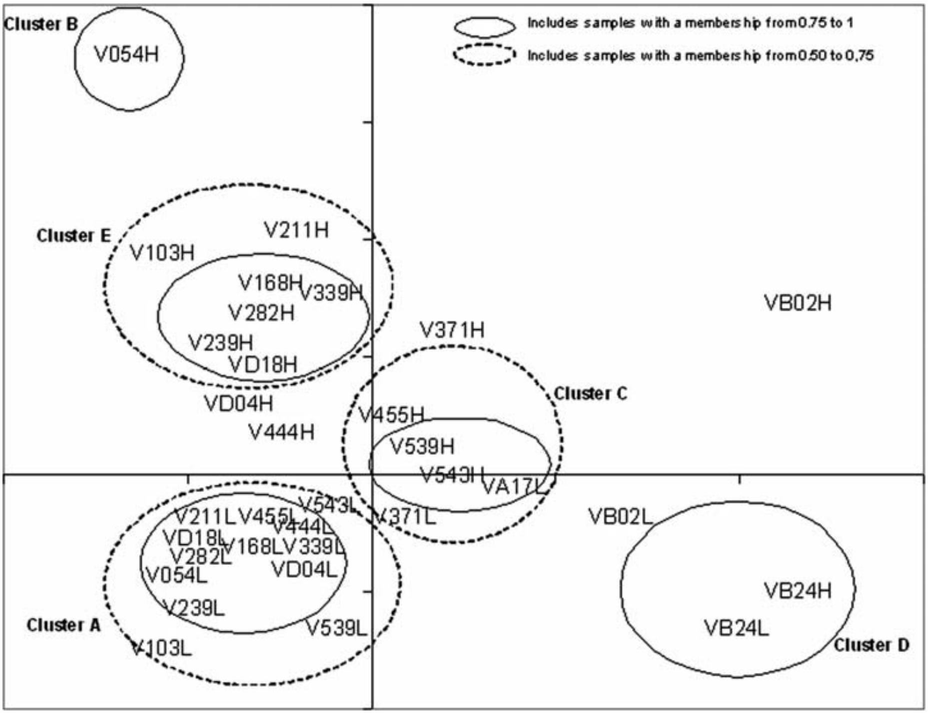

Figure 5. Classification of the Aoos-Vjose river sites with the FUZZY method based on their benthic macroinvertebrate fauna. Samples taken during the high flow period (Spring 2001) are marked with an H, whereas during low flow period (Autumn 2001) with an L.

Table 5. Simper Average Similarity and Dissimilarity between the five clusters formed by FUZZY. The clustering is based on the benthic macroinvertebrate community found at the 17 sampling sites along Aoos-Vjose river during Spring and Autumn 2001.

Average Similarity%	**Cluster A** **56.75**	**Cluster B** **100.00**	**Cluster C** **48.35**	**Cluster D** **67.23**	**Cluster E** **60.71**
Average Disimilarity%					
Cluster A	–				
Cluster B	80.28	–			
Cluster C	54.20	83.58	–		
Cluster D	60.73	92.38	55.24	–	
Cluster E	53.47	72.79	53.97	65.35	–

4. Discussion

In this paper we assessed the ecological integrity of a large transboundary South Balkan river through the combined evaluation of the river's physicochemical, biotic and habitat elements. Our results indicate that Aoos-Vjose River can be regarded as predominantly undisturbed, almost unpolluted, with high habitat quality and a rich macrobenthos community.

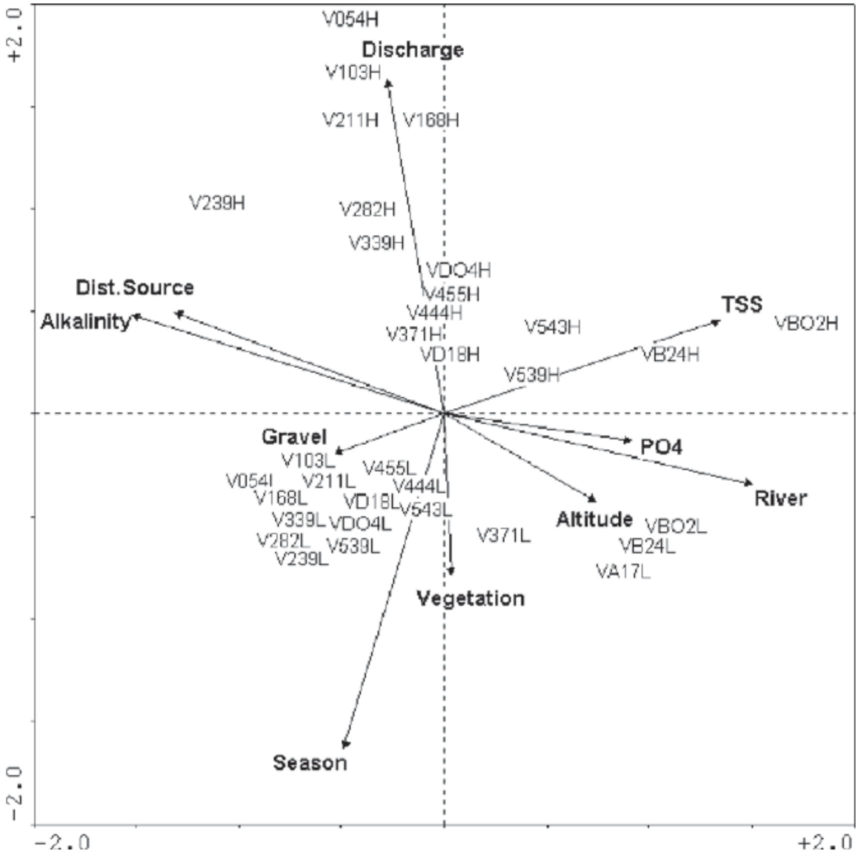

Figure 6. Canonical Correspondence Analysis (CCA) diagram with 10 significantly contributing environmental variables and 33 numbered samples of the 17 sites from the Aoos-Vjose River. Samples taken during high flow period (Spring 2001) are marked with an H, whereas during low flow period (Autumn 2001) with an L.

The high biotic HBMWP and HASPT scores indicate the existence of macrobenthos communities sensitive to pollution. Consequently, the water quality was in most cases good. This result is in line with our observations which indicate that the overall physicochemical character of Aoos-Vjose is mainly governed by natural processes. The high values of total dissolved solids and conductivity are mainly attributed to the high calcium and bicarbonate content of the underlying geology (SKOULIKIDIS *et al.*, 1998). Total suspended solids levels are similar to the ones measured in other rivers in North Greece (LAZARIDOU–DIMITRIADOU *et al.*, 2000; KAMPA *et al.*, 2000), being primarily a product of the weathering processes taking place in the naturally vulnerable to erosion Mediterranean landscape (WOODWARD, 1995).

The evaluation of the extent of human impact qualified 16 of our 17 surveyed sites either as pristine (semi- natural) or predominantly unmodified, indicating that Aoos-Vjose River channel has not been subjected to major human interference. Similarly, by comparing Aoos-Vjose's HQA scores with the rest from our South Balkan rivers database (CHATZINIKOLAOU *et al.*, 2006), we conclude that the river is characterized by a highly diverse instream environment. Such physical diversity, although not directly related to rich benthic communities (TICKNER *et al.*, 2000), may imply the influence of positive riverine processes on water quality (Bis *et al*., 2000). The unregulated state of the Aoos-Vjose River allows the free development of geomorphologic features (such as riffles, pools, extensive braided channels, lateral connectivity) which enhance self-purification and retention processes (Heidenwag *et al*., 2001). We assume that such processes may explain the quick reduction of nutrient concentrations downstream of point sources of untreated sewage effluents from small towns in the Albanian part of the river.

Habitat modification is significantly negatively correlated ($P < 0.01$) with HBMWP and taxa richness, implying that the more modified a river is, the less macrobenthos diversity is expected. Thus, in Aoos-Vjose River the lack of severe anthropogenic alterations of habitat (dams, weirs, embankment, realigned channel *etc*.), together with its good water quality conditions imply that parts of the river can be validly considered as reference sites.

In absence of pollution and habitat modifications, changes in benthic macroinvertebrate community composition along the river gradient indicate the ability of species to occupy different microhabitats, since their distribution is influenced by their response to substrate composition (Buss *et al*., 2002), water chemistry (Basaguren *et al*., 1996) and hydraulic conditions (Hynes, 1970; Gilvear *et al*., 2002). In the present study we verified these relationships. CCA correlated axis I to alkalinity, river tributary and distance from source. Alkalinity is basically influenced by its underlying geology and therefore axis I can be interpreted as the spatial information axis. Axis II is best correlated to season and discharge; therefore, it can be interpreted as the seasonal axis. These results are in agreement with Gasith and Resh (1999), who claim that in Mediterranean rivers seasonality and the river section are the principal attributes of the system and support the conclusion that the benthic communities of Aoos-Vjose River are least affected by anthropogenic impacts.

A scheme of assessing the ecological integrity of a river course based on the interplay of environmental variables with benthic macroinvertebrates is proposed by the current study. Multivariate statistics sufficiently explained the seasonal and spatial patterns of macrobenthos composition, even if we have neglected other aspects of biotic interactions (like competition or predation), whereas both biotic and habitat indices showed that the water and river habitat are of good quality. Based on these observations, this study offers insight into the structure of macroinvertebrate communities in an almost intact large transboundary Mediterranean river and it constitutes "baseline" information to be used for comparing the ecological quality of other major rivers of the same type in the East Mediterranean region.

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