

Impact of a reservoir system on benthic macroinvertebrate and diatom communities of a large Mediterranean river (lower Ebro river, Catalonia, Spain)

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ABSTRACT

Impact of a reservoir system on benthic macroinvertebrate and diatom communities of a large Mediterranean river (lower Ebro river, Catalonia, Spain)

This study included the main hydrogeomorphic categories of the lower Ebro River and aimed to detect spatial and temporal patterns of macroinvertebrates and diatoms communities inhabiting downstream a reservoir system. We hypothesized that biological communities will reflect a gradient of alteration related to the distance from the impact (i.e., dams). Surveys conducted along five river sections 2 km long integrating different years and seasons were analyzed. Non-metrical Multidimensional Scaling (MDS), Similarity Percentage Analysis (SIMPER) and 1-way Analysis of Similarities (ANOSIM) were performed to assess spatial and temporal differences in community structure. The relationship between biological and environmental data was investigated with BIOENV routine and a Principal Components Analysis (PCA) was also carried out. Significant differences in the composition of the macroinvertebrate community were found between the section closest to reservoirs (E5) and the rest of the study sections; the present community was also very different of that found in previous studies carried out in the 80's and 90's. For the case of diatoms, significant differences in community composition were only found between seasons but not between study sections. As well, indices for ecological status assessment based on both macroinvertebrates (IBMWP) and diatoms (IPS) showed lowest scores at section E5, likely caused by the impact of dams. Macroinvertebrates and diatoms responded rather differently to anthropogenic stressors in the lower Ebro River; macroinvertebrates were more sensitive to hydromorphological impacts, while diatoms were more sensitive to water quality alteration.

Key words: benthos, large Mediterranean river, damming, ecological status, Water Framework Directive

RESUMEN

Impacto de un sistema de embalses sobre las comunidades bentónicas de macroinvertebrados y diatomeas de un gran río Mediterráneo (bajo Ebro, Cataluña, España)

El presente estudio incluyó las principales categorías hidrogeomorfológicas de la parte baja del río Ebro y tuvo como objetivo detectar los patrones de distribución de macroinvertebrados y diatomeas bentónicas aguas abajo de un sistema de embalses. Se estableció la hipótesis que las comunidades biológicas reflejan un gradiente de alteración relacionado con la distancia al impacto (por ejemplo, las presas). Se analizaron muestreos realizados a lo largo de cinco tramos del río de 2 Km, integrando diferentes años y estaciones. Diversos análisis estadísticos (MDS, SIMPER, ANOSIM) fueron utilizados para evaluar las

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diferencias espaciales y temporales en las comunidades biológicas. También se analizó la relación entre los datos biológicos y ambientales con el test BIOENV y los patrones de asociación de las variables abióticas se analizaron a través de un PCA. Se encontraron diferencias significativas en la composición de las comunidades de macroinvertebrados entre la sección más cercana a los embalses y el resto de las secciones de estudio; la composición actual de éstas comunidades fue muy diferente de la encontrada en estudios de los años 80-90. En el caso de las diatomeas, solamente se encontraron diferencias significativas a nivel estacional, pero no entre secciones de estudio. Además, los índices para la evaluación del estado ecológico basados en macroinvertebrados (IBMWP) y diatomeas (IPS) mostraron puntuaciones más bajas en la sección E5, posiblemente como resultado del impacto de las presas. Los macroinvertebrados y las diatomeas respondieron de forma diferente a los factores antropogénicos en el tramo final del río Ebro; los macroinvertebrados fueron más sensibles a los impactos hidromorfológicos, mientras que las diatomeas fueron más sensibles a las alteraciones de calidad del agua.

Palabras clave: bentos, gran río Mediterráneo, embalse, estado ecológico, Directiva Marco del Agua

INTRODUCTION

Mediterranean climate regions are hotspots of biodiversity widespread in several continents and supporting similar types of ecosystems characterized by strong spatial, seasonal and year to year variation (Kondolf *et al.*, 2012; Stamou *et al.*, 2004). Some of these regions share as a common factor the presence of large Mediterranean rivers (e.g. Ebro in Spain, San Joaquin and Sacramento in USA, Biobío in Chile) with comparable structural and functional features and similarly influenced by climatic and geomorphic settings (Fisher, 1995; Gasith & Resh, 1999; Gushing *et al.*, 1995; King *et al.*, 1988; Puckridge *et al.*, 1998).

Large Mediterranean rivers have been extensively dammed during the last century and reservoirs have been built to regulate the variability in water supplies for agricultural irrigation and power generation (Kondolf *et al.*, 2012). In fact they are considered to be subject to perhaps the highest levels of water infrastructure development in the world (Grantham *et al.*, 2013). However, while streams are the most studied component of Mediterranean (and world) rivers, there is fewer research involving large rivers due to the required sampling effort, economic cost of research, methodological problems for sampling and scarcity of this type of rivers.

Biomonitoring protocols based on benthic communities are widely used for running waters worldwide (Furse, 2006; Kelly & Whitton, 1998; Metcalfe, 1989; Quevauviller *et al.*, 2008) and, benthic macroinvertebrates and diatoms are frequently used as bioindicators of the ecological status. By analyzing these two biological groups, both long-term and short-term changes of environ-

mental conditions can be detected (Li et al., 2010).

Diatoms have been also used for river biomonitoring purposes by many authors (Chessman et al., 1999; McCormick & Cairns Jr, 1994; Whitton et al., 1991), and several biotic indices have been successfully applied to estimate the status of river ecosystems (Eloranta & Soininen, 2002; Goma et al., 2005; Kelly et al., 2009; Prygiel & Coste, 1993). Macroinvertebrates also have been widely reported as bioindicators for aquatic ecosystems (Buffagni et al., 2004; Lafont, 2011; Rosenberg & Resh, 1993; Statzner et al., 2001; Vivas et al., 2002; Oliveira & Cortes, 2005; Varandas & Cortes, 2010; Cortes et al., 2013) as well as indicators for the assessment of anthropogenic hydrological alterations (Dunbar et al., 2010; Gore et al., 2001; Lafont et al., 2010; Suren & Jowett, 2006; Vivas et al., 2002). Furthermore, in several studies (Johnson et al., 2006; Soininen & Könönen, 2004; Torrisi et al., 2010; Tupinambás et al., 2014), macroinvertebrate and diatom communities have been used together comparing different assemblage responses to different anthropogenic pressures.

The European Union has led efforts to incorporate protection to these aquatic ecosystems through the expedition of the Water Framework Directive WFD 2000/60/EC (European Commission, 2000) which commits to state members to achieve a good ecological status of water bodies. One of the criteria established for this purpose is the implementation of biomonitoring programs based on biological quality elements, including diatoms and macroinvertebrates as central elements for ecological quality assessment.

The Ebro is the largest river in Spain in terms of water discharge, and agriculture is the domi-

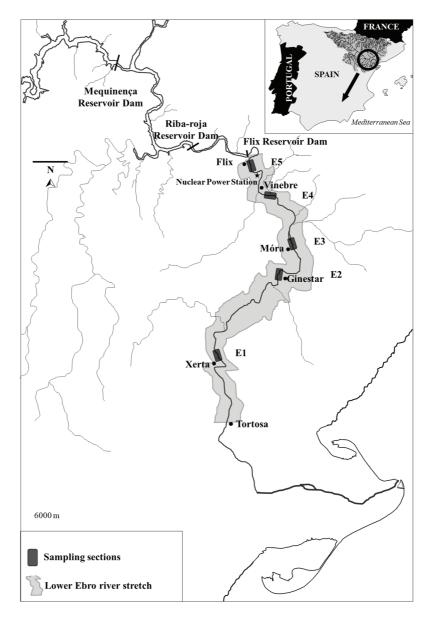


Figure 1. Map of the lower Ebro River showing the study area and the location of sampling sections. *Mapa del bajo Ebro mostrando el área de estudio y la localización de las secciones de muestreo*.

nant land use of its basin (Grantham *et al.*, 2013). The lower part is regulated by a system of three reservoirs (Mequinensa, Riba-roja and Flix) which heavily modified the river hydrology, geomorphology and ecology by altering the magnitude, the timing and duration of flows, the sediment dynamics, the water temperature regime and the geochemistry (Ibáñez *et al.*, 1996). In the Ebro River, benthic communities have been used

to analyze the effects of chemical pollution, flow regulation and eutrophication (Cid *et al.*, 2010; Muñoz & Prat, 1996; Tornés *et al.*, 2007) and to assess the ecological status of the river (Oscoz *et al.*, 2007), but in the lower Ebro most of the ecological studies have focused on the estuary area (Nebra *et al.*, 2011; Rovira *et al.*, 2009; Rovira *et al.*, 2012a; Rovira *et al.*, 2012b).

The Catalan Water Agency (ACA) and the

Hydrographical Confederacy of the Ebro (CHE) have developed periodical monitoring programs based on benthic communities (available in http://www.chebro.es and http://aca-web.gencat.cat) to determine water quality and to assess the ecological status; however, these reports do not include information neither about community structure nor the response of the communities to anthropogenic pressures (e.g. water regulation system); furthermore, most of the monitoring points are located upstream of Mequinensa and Riba-roja dams, even though, the lower part of the river evidence in great measure the consequences of the factors exerting pressure upstream.

This study aimed to detect spatial and temporal patterns of macroinvertebrates and diatom communities in the lower Ebro downstream a reservoir system, and to investigate the environmental (i.e., substrate, water quality) and anthropogenic (i.e., distance to dams) factors influencing these communities, in order to understand the ecology of large Mediterranean rivers strongly influenced by dams and other human pressures (i.e., chemical and thermal pollution). We hypothesize that biological communities will reflect a gradient of alteration related to the distance from the impact.

MATERIAL AND METHODS

Study area

The Ebro basin is located in the NE part of the Iberian Peninsula (Fig. 1), with a surface of 85 534 km². The Ebro River it is one of the most important tributaries to the Western Mediterranean Sea. The main river is 928 km long and its principal tributaries are the Segre, Aragón, Cinca, and Gállego rivers. The climate is continental in most of the basin with a transition from mountain climate at the north (Pyrenees) to Mediterranean climate at the lower part. The basin has been strongly regulated by nearly 190 dams (Batalla et al., 2004) and the main land use is agriculture, which accounts for approximately 90 % of water usage for irrigating more than 1 million hectares (Ibáñez et al., 2008). The mean annual flow near the upper end of the estuary (Tortosa) was 592 m³/s at the beginning of the 20th century but, increasing water uses has led to a decreasing tendency since the 70's, down to about 400 m³/s (Ibáñez et al., 1996). In the lower Ebro there are two large reservoirs, (Mequinensa and Riba-roja) built in 1964 and 1969 respectively for hydropower purposes (Ibáñez et al., 1996); downstream Riba-roja, a small reservoir (Flix) and a nuclear power station (Ascó, operating since 1984) are located. A concession of 72.3 m³/s of the Ebro's flow is granted to the power station for the cooling system, that returns water 2-3 °C warmer (Prats et al., 2010). The Flix reservoir contains a large amount of sediments polluted with heavy metals such as mercury, organochlorine compounds and other toxics accumulated along 70 years due to the presence of a chemical factory (Cid et al., 2010).

This study was performed in an area (Fig. 1) that extends from the reservoir furthest downstream (Flix) to the upper limit of the estuary (Tortosa). In the study area the river is about 80 km long, 150 m wide, 5 m deep and the substrate is dominated by gravels.

Sampling Sites

Five (E1 to E5) sampling sections (Fig. 1) were randomly selected to perform the surveys in order to cover all the lower Ebro hydrogeomorphic variability. For this purpose aerial photographs were analyzed in order to classify the lower Ebro River in different morphological categories according to a simplified classification (Rosgen, 1994). Four morphological river categories were identified in the study area: steep stretches, moderately entrenched stretches, entrenched meanders and low gradient meanders. Then, a polyline GIS layer representing the lower Ebro River was classified according to these four morphological river categories and transformed into a point GIS layer, with equidistant points (1 Km). Thus, the aforementioned 5 sampling sections were selected in order to ensure the coverage of the morphological variability (see Caiola et al., 2014).

Benthic surveys covering different years and seasons were conducted between 2006 and 2009, resulting in a total of 31 and 20 sampling occasions for macroinvertebrates and diatoms, respectively. Unfortunately, for logistic reasons diatoms were

not sampled on every occasion that macroinvertebrates were collected, and never in winter. For every sampling site and occasion, physicochemical data and hydromorphological characteristics were recorded. Water velocity at 60 % of total water depth was recorded with a Braystoke BFM 001 current meter; an YSI 556 multi-parameter probe was used to measure water temperature (°C). dissolved oxygen (mg/l), oxygen saturation (%), pH, salinity (ppt) and conductivity (mS/cm). Water depth (m) was measured using a Speedtech SM-5 depth-meter sounder. Substrate composition analysis was based on Wentworth (1922) scale including the following fractions: sand (<2 mm), gravel (2-16 mm), pebble (16-64 mm), cobble (64-256 mm), boulder (>256 mm) and was determined by a visual assessment of the distribution of particle size classes. Analysis of total dissolved nitrogen (TDN), soluble reactive phosphorus (SRP) and SiO₄ were measured according to Koroleff (1977). Finally, the total of planktonic chlorophyll concentration was calculated using the colorimetric method (Jeffrey & Humphrey, 1975).

Macroinvertebrate sampling

Benthic macroinvertebrates were collected in the littoral zone; sampling the central part of the river channel with a drag was not possible due to high water velocity and type of substrate (coarse gravel). The riverbed was disturbed and organisms were captured using a Surber net with a mesh size of 500 µm and preserved in 4 % formaldehyde. Each sample was composed by fauna collected in at least three locations along each sampling section. At the laboratory, samples were rinsed in a 500 µm mesh to remove fine sediments and coarse organic material (whole leaves, twigs, algal and macrophyte mats) was thoroughly rinsed, visually inspected, and discarded. Then, the sample was evenly distributed in a flat pan of 30x36 cm marked with a numbered grid pattern of 6x6 cm, and a random number table was used to select squares in order to get subsamples. The content of the selected square was transferred to a Petri dish, sorted and identified under an apochromatic corrected stereomicroscope Leica M165C with 16.5:1 zoom and maximum 906 Lp/mm. Additional squares were added to the subsample until obtaining a minimum of 200 organisms.

Macroinvertebrates were identified according to Müller-Liebenau (1969), Nocentini and Delle Ricerche (1985), Rossaro (1982), Tachet *et al.* (2000) and Vieira (2000) at genus level, except for Oligochaeta which were kept at sub class level and some Diptera which were kept at sub family or tribe level. Each species was classified into feeding guilds based on Tachet *et al.* (2000). The feeding guilds included were the following: Absorber (A), Deposit feeder (D), Shredder (Sh), Scraper (Sc), Filter-feeder (F), Piercer (P), Predator (Pr) and Parasite (Ps).

Diatom sampling

Benthic diatom samples were collected from submerged natural substrata (stones) by brushing their top surfaces according to the recommendations of Kelly et al. (1998). Each sample was a composite drawn from at least three stones. The suspension was fixed in 4 % formaldehyde solution. At the laboratory, diatom samples were oxidized with H₂O₂ 30 % V/V for several hours in order to remove the organic matter. HCl⁻ 37 % V/V was added to evaporate the carbonates from the samples, as described in Renberg (1990). Clean valves were permanently mounted with Naphrax[©] (refractive index 1.74). The permanent slides were examined using a LEICA DMI 3000 B light microscope equipped with differential interference contrast (DIC) with a 100 times oil immersion objective (n.a = 1.40). For each sampling occasion the samples collected from each river section were processed and a minimum of 400 valves were counted each time. Identification of diatoms was done to species level mainly Krammer and Lange-Bertalot following (1986-1991) but other taxonomic and floristic works were also used when needed.

Data analysis

For both biological groups (macroinvertebrates and diatoms), descriptive community parameters were calculated for each river section: Richness (S), Shannon-Wiener's diversity index (H', as log₂) and Pielou's evenness index (J'). Ecological

Table 1. Values of physicochemical parameters measured at each sampling section: Dist= distance to the dam, T=temperature, DO = dissolved oxygen, Cond = conductivity, Sal= salinity, SPR = soluble reactive phosphate, TDN = total dissolved nitrogen, TN = total nitrogen, Chl a = chlorophyll a. Valores de los parámetros físico-químicos medidos en cada sección de muestreo: Dist= distancia a la presa, T=temperatura, DO = oxígeno disuelto, Cond=conductividad, Sal=salinidad, SPR=fosfato reactivo soluble, TDN=nitrógeno total disuelto, TN=nitrógeno total, Chl a=clorofila a.

	Dist	T	рН	DO	DO	Cond	Sal	SRP	TDN	SiO ₄	Chl a	Depth		Cobble		Gravel	
	(m)	(°C)	P	(mg/l)	(%)	(mS/cm)	(ppt)	(mg/l)	(mg/l)	(mg/l)	(µg/l)	(m)	(m/s)	(%)	(%)	(%)	(%)
	Spring																
E1	59329.03	17.55	8.01	9.51	99.85	894.30	0.53	0.02	2.31	0.87	0.69	0.85	0.34	00	67	33	00
E2	35061.43	19.18	8.00	9.09	98.63	704.33	0.39	0.02	1.96	0.68	0.45	0.97	0.34	12	74	14	00
E3	23998.65	18.06	7.99	9.82	104.00	873.60	0.51	0.02	1.81	0.80	0.73	0.87	0.28	10	48	30	12
E4	17575.58	20.37	8.06	8.80	97.73	674.00	0.36	0.02	1.53	0.63	1.08	0.94	0.03	03	46	35	16
E5	7858.15	15.39	7.93	8.73	87.78	818.02	0.51	0.01	2.38	0.75	1.46	0.66	0.17	44	39	17	00
	Summer																
E1	59329.03	24.60	8.19	7.27	87.78	978.55	0.49	0.05	1.62	0.67	1.20	0.73	0.36	00	67	33	00
E2	35061.43	25.24	8.32	6.70	81.80	1341.50	0.67	0.03	1.33	0.75	1.07	0.97	0.34	12	74	14	00
E3	23998.65	24.85	8.06	6.82	82.46	975.68	0.49	0.04	1.55	0.64	0.84	0.80	0.38	10	48	30	12
E4	17575.58	25.73	8.42	7.62	93.90	1348.90	0.66	0.02	1.35	0.71	1.06	0.94	0.03	03	46	35	16
E5	7858.15	22.56	7.93	6.88	80.24	943.41	0.49	0.04	1.68	0.66	1.34	0.65	0.18	44	39	17	00
	Autumn																
E1	59329.03	21.50	8.33	7.85	89.30	1456.33	0.79	0.03	1.91	0.53	1.07	1.03	0.31	00	67	33	00
E2	35061.43	22.58	8.34	9.08	105.43	1474.00	0.78	0.04	2.46	0.39	0.59	0.97	0.34	12	74	14	00
E3	23998.65	22.21	8.23	7.88	90.90	1463.33	0.78	0.04	2.50	0.52	0.88	1.02	0.35	10	48	30	12
E4	17575.58	22.58	8.34	9.08	105.43	1474.00	0.78	0.04	2.49	0.55	1.45	0.94	0.03	03	46	35	16
E5	7858.15	21.50	8.33	7.85	89.30	1456.33	0.79	0.04	2.53	0.44	2.03	0.66	0.17	44	39	17	00
	Winter																
E1	59329.03	12.74	8.18	10.05	95.33	995.00	0.51	0.09	2.46	0.03	0.80	1.03	0.31	00	67	33	00
E2	35061.43	12.31	8.19	9.88	92.07	1006.50	0.52	0.08	2.96	0.06	0.78	0.97	0.34	12	74	14	00
E3	23998.65	12.30	8.19	10.14	95.39	1006.50	0.52	0.04	2.89	0.13	1.01	1.02	0.35	10	48	30	12
E4	17575.58	11.60	8.16	10.23	94.75	1006.50	0.52	0.11	3.19	0.06	0.86	0.94	0.03	03	46	35	16
E5	7858.15	9.75	8.12	10.56	93.69	1018.00	0.52	0.04	3.09	0.09	0.60	0.66	0.17	44	39	17	00

status was estimated by calculating the official indices used in Mediterranean rivers in Spain according to the WFD; these were IBMWP (Iberian Biological Monitoring Working Party) (Alba-Tercedor & Sánchez-Ortega, 1988) for macroinvertebrates and IPS (Specific Polluosensitivity Index) for diatoms calculated by using the software OMNIDIA (Lecointe *et al.*, 1993).

To avoid the effect of rare species, only species with a relative abundance higher than 0.05 % for diatoms and 0.02 % for macroinvertebrates were included in the analysis of each sample. Then, abundance data was square-root transformed in order to downweight the contribution of the most abundant taxa and similarity matrices were computed using the Bray-Curtis coefficient (Legendre & Legendre, 1998). All environmental variables that expressed concentration were log-transformed and statistical analyses were performed using the different routines available in the Multivariate Ecological Research Software Package PRIMER V6 (Clarke

& Gorley, 2006).

For diatoms and macroinvertebrates separately, samples and taxon abundances were ordered using Non-metrical Multidimensional Scaling (MDS) and significant differences in assemblages composition among sections and seasons were identified using 1-way Analysis of Similarities test (ANOSIM), that hypothesizes for differences between groups of samples (defined a priori) through randomization methods on a resemblance matrix. Then, in order to identify resemblances between sample groups and to identify taxa that contributed to dissimilarity among sections, a Similarity Percentage Analysis (SIM-PER) was performed.

The relationship between the community structure and environmental variables was investigated with the BIOENV routine (Clarke & Ainsworth, 1993; Clarke & Warwick, 2001), which maximizes a rank correlation (Spearman's coefficient) between resemblance matrices derived from biotic and environmental data, iterat-

ing for all possible combinations of environmental variables. A Spearman's coefficient value close to 0 indicates a weak relation between the community and environmental variables, whereas a value close to 1 indicates that the selected environmental variables explain the variability of the community structure. Finally, in order to illustrate patterns of association among the environmental variables, two Principal Component Analyses (PCA) were carried out with the environmental data of each biological group (because the sampling occasions were not fully coincident).

RESULTS

Environmental parameters

The average values of environmental parameters measured at each sampling section are shown in Table 1. During the study period temperature ranged between 9.7 °C (E5, winter) and 24.8 °C (E3, summer), and was lower in the uppermost section (E5) which is located upstream the Ascó nuclear power station and right downstream Flix dam. Dissolved oxygen showed highest values in spring (E3, 104 %) and lowest in summer (E5, 80 %) in the uppermost section; pH showed the highest and lowest values in summer (E4, 8.42; E5,

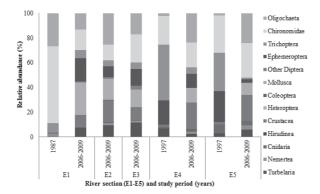


Figure 2. Macroinvertebrate community structure (% abundance) per section in the period 2006-2009 (this study), and comparison with previous studies carried out in sections E1 (Muñoz & Prat, 1994), E4 and E5 (LIMNOS, 1997). *Estructura de la comunidad de macroinvertebrados (% de abundancia) por sección en el período 2006-2009 (presente estudio), y comparación con previos estudios llevados a cabo en las secciones E1 (1987), E4 y E5 (1997).*

7.93); conductivity showed a minimum in spring (E4, 674 mS/cm) and a maximum in autumn (E4, 1474 mS/cm); total dissolved nitrogen showed a maximum value of 3.09 mg/l (E5, winter) and a minimum of 1.33 mg/l (E2, summer); soluble reactive phosphorus showed a minimum of 0.01 mg/l (E5, spring) and a maximum of 0.11 mg/l (E4, winter); SiO4 ranged from 0.03 mg/l (E1, winter) to 0.87 mg/l (E1, spring); water chlorophyll varied from 0.45 μg/l (E2, spring) to 2.03 μg/l (E5, autumn). E5 section showed the coarsest substrata composition (44 % cobble) in relation to sections downstream (E1 E2 E3 E4), where pebbles where the dominant fraction.

Macroinvertebrate assemblages

During the sampling period a total of 66 430 individuals were collected belonging to 46 different taxa that comprised 37 genus and 36 families (Table S1, http://www.limnetica.net/en/limnetica). Artropoda was the dominant phylum and accounted for 67.78 % of the total abundance. Mollusca and Anellida contributed with 11.44 % and 11.32 %, respectively. Chironomidae (28.19 %), Gammaridae (15.72 %) and Baetidae (9.06 %) were the most abundant families. Most of the taxa found belong to Insecta and includes: 6 mayflies (Ephemeroptera), 7 caddisflies (Trichoptera), 2 aquatic beetles (Coleoptera), 2 dragonfly and damselfly (Odonata), 10 true flies (Diptera), 1 net-winged insects (Neuroptera) and 1 true bug (Hemiptera). Macroinvertebrate community structure in each section, including a comparison with previous data available for E1, E4 and E5 is shown in (Fig. 2).

Regarding macroinvertebrate diversity (Table 2), the highest values of species richness were found in summer (E4, 27 taxa) and the lowest number of species (12 taxa) occurred in spring (E5) and winter (E4).

The mean values for IBMWP showed different seasonal ranges: in spring values ranged from 29 to 84, in summer fluctuated between 52 and 112, in autumn from 37 to 60 and during winter between 42 and 83. The highest value was found in summer at E4 indicating "very good" ecological status, whereas the lowest value was registered in spring at E5 indicating "poor" ecological status (Table 2).

Table 2. Macroinvertebrate community descriptive parameters for each sampling section. Richness (S), Shannon-Wiener's diversity index (H', as log2) and Pielou's evenness index (J'). Includes value and category of IBMWP index (Iberian Biological Monitoring Working Party). Parámetros descriptivos de la comunidad de macroinvertebrados de cada sección de muestreo. Riqueza (S), índice de diversidad de Shannon-Wiener (H', as log2) e índice de equidad de Pielou (J'). Se incluye el valor y la categoría del índice IBMWP.

	S	H'(log ₂)	J'	IBMWP			
Spri	ng	-		Value	Category		
E1	19	2.39	0.56	84	Good		
E2	17	1.54	0.38	55	Moderate		
E3	21	2.47	0.56	85	Good		
E4	14	1.87	0.49	40	Moderate		
E5	12	1.98	0.55	29	Poor		
Summer							
E1	19	3.00	0.71	82	Good		
E2	20	3.37	0.78	82	Good		
E3	20	2.91	0.68	87	Good		
E4	27	3.70	0.78	112	Very good		
E5	17	2.73	0.67	52	Moderate		
Auti	umn						
E1	13	2.09	0.56	37	Moderate		
E2	17	2.55	0.62	60	Moderate		
E3	16	2.16	0.54	56	Moderate		
E4	15	2.50	0.64	49	Moderate		
E5	17	2.29	0.56	51	Moderate		
Winter							
E1	14	2.75	0.72	43	Moderate		
E2	21	2.55	0.58	83	Good		
E3	18	3.22	0.77	70	Good		
E4	12	2.78	0.78	42	Moderate		
E5	15	2.85	0.73	51	Moderate		

In terms of trophic structure, the dominant feeding guilds were scrappers (48.52 %) followed by shredders (24.28 %) and deposit feeders (20.48 %). Seasonal changes due to the inter-annual variability showed a dominance of scrapers during spring (32.64 %) and summer (42.89 %), of shredders in winter (38.41 %), and deposit feeders in autumn (47.04 %). Table S1 provides a

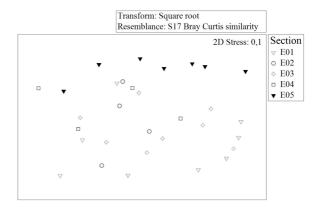


Figure 3. Two dimensional MDS plots based on Bray-Curtis similarities of square-root transformed macroinvertebrate abundance data. Samples are ordered by sites (sections). *Gráfico Bidimensional MDS basado en similitudes Bray-Curtis de datos transformados a la raíz cuadrada de la abundancia de macroinvertebrados. Las muestras se ordenan por sitios (secciones).*

list of macroinvertebrate taxa found over the study period including feeding guilds and the sections where each taxon was found.

Macroinvertebrate MDS analysis (Fig. 3) showed two different communities, one corresponding to the section located next to the Flix dam (E5) and another that included sections located downstream (E1 E2 E3 E4), hereafter E1-4. Significant differences in community composition were found between E5 and E1-4 (ANO-SIM r: 0.218, p=0.01), and also among seasons (ANOSIM r: 0.433, p=0.001) (spring \neq summer \neq autumn \neq winter). Fig. 3 shows the MDS ordination as a function of study sites (sections).

Similarity Percentages analysis SIMPER (Table S2, http://www.limnetica.net/en/limnetica) showed that the mean community similarity within E5 group was 42.57 %, and taxa that most contributed to the high similarity were Oligochaeta (21.75 %), Orthocladiinae (15.62 %), Echinogammarus (15.08 %) and Proasellus (8.77 %); a total of 11 taxa were necessary to accumulate 90 % of similarity. The mean similarity within E1-4 group was 40.98 % with a high contribution of Oligochaeta (15.84 %), Orthocladiinae (14.27 %), Corbicula (13.47 %), Echinogammarus (9.46 %) and Baetis (9.15 %); 90 % of similarity in this group was obtained with 14 taxa. Furthermore the mean dissimilarity between these two groups was 64.54 % with Orthocladiinae, Oligochaeta, *Echinogammarus*, *Micronecta*, *Dugesia*, *Baetis* and *Corbicula* as the taxa with the highest contributions to dissimilarity.

BIOENV analysis showed that the combination of water temperature, substrate composition, dissolved oxygen, pH, conductivity and distance to the dam had the strongest influence on the structure of macroinvertebrate communities (p =0.378). The two first axis of the PCA (Fig. 4) explained 65.4 % of the total variance. The first axis (37.5 %) summarized variables displaying spatial and substrate variation, where the distance to the dam was inversely related to substrata size, which is related to the influence of the dam regulation system. The second axis (27.9 %) is likely associated to the seasonal variation, where water temperature was opposed to dissolved oxygen levels, but could be also related to the spatial variation due to the presence of dams and the nuclear power station. Fig. 4 shows the PCA ordination as a function of study sites (sections).

Diatom assemblages

A total of 124 diatom species were found during the study period. Only 67 had a relative abundance higher than 0.05 % and therefore were used in the statistical analyses and are listed in Table S3, http://www.limnetica.net/en/limnetica. Some species were present in all samples and abundant

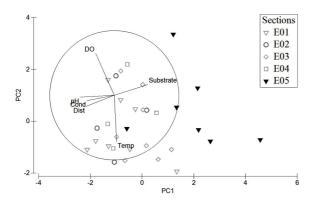


Figure 4. PCA of the macroinvertebrate data showing the ordination of sampling sections as a function of the environmental variables. Samples are ordered by sites (sections). PCA de datos de macroinvertebrados mostrando la ordenación de las secciones de muestreo en función de las variables ambientales. Las muestras se ordenan por sitios (secciones).

in many of them (e.g. Amphora pediculus, Cocconeis placentula var. lineata, Nitzschia dissipata var. dissipata, N. inconspicua and N. Palea). Others, such as Navicula antonii and N. cryptotenella, though rarely exceeding 10 % relative abundance, were also present throughout. Significant changes occurred in the diatom community along the year: in summer, communities were mainly dominated by Cocconeis placentula var. lineata and Nitzschia palea, and in autumn by these two species as well as Amphora pediculus. In spring the diatom community was mainly dominated by A. pediculus, Nitzschia dissipata var. dissipata and N. inconspicua.

Concerning diatom diversity (Table 3), there was no clear pattern among river sections. Howev-

Table 3. Diatom community descriptive parameters for each sampling section. Richness (S), Shannon-Wiener's diversity index (H', as log2) and Pielou's evenness index (J'). Includes value and category of IPS index (Specific Polluosensitivity Index). Parámetros descriptivos de la comunidad de diatomeas de cada sección de muestreo. Riqueza (S), índice de diversidad de Shannon-Wiener (H', as log2) e índice de equidad de Pielou (J'). Se incluye el valor y la categoría del índice IPS.

	S	H'(log ₂)	.J'		IPS			
	3	11 (10g ₂)	J	** 1				
Spring				Value	Category			
E1	27	3.45	0.74	14.40	Good			
E2	29	3.32	0.68	13.90	Good			
E3	22	2.62	0.58	14.87	Good			
E4	23	3.33	0.74	13.70	Good			
E5	26	2.88	0.62	12.40	Moderate			
Summer								
E1	29	3.75	0.77	12.00	Moderate			
E2	25	3.21	0.69	10.90	Moderate			
E3	30	4.12	0.84	12.90	Moderate			
E4	28	3.52	0.73	11.50	Moderate			
E5	34	4.18	0.82	10.40	Moderate			
Autumn								
E1	36	3.69	0.71	11.90	Moderate			
E2	32	3.74	0.75	12.10	Good			
E3	32	2.72	0.54	13.70	Good			
E4	35	3.66	0.71	10.50	Moderate			
E5	31	3.49	0.71	8.70	Poor			

er, there seemed to be a slight seasonal pattern with higher species richness in autumn. The lowest number of species occurred in spring (22 in E3).

Diatom MDS analysis showed a defined pattern of distribution at seasonal scale (Fig. 5). and significant differences were found among seasons (ANOSIM r: 0.549, p=0.001) (spring \neq summer \(\neq \) autumn) but not among sections (ANOSIM r: -0.122, p=0.91). BIOENV analysis (ρ=0.482) showed a significant relation between the diatom community distribution and the combination of the following environmental variables: water temperature, pH, dissolved oxygen, soluble reactive phosphorus, SiO₄, chlorophyll and total dissolved nitrogen. The two first axis of the PCA (Fig. 6) explained 61.2 % of total variance; the first axis (40.9 %) summarized variables representing the seasonal variation, being water temperature opposed to dissolved oxygen and total dissolved nitrogen; and the second axis (20.3 %) summarized the effects of dam regulation on water quality, displaying SiO₄ opposed to soluble reactive phosphorus, pH and chlorophyll. Fig. 5 and 6 show the MDS and PCA ordinations as a function of seasons, since there were not differences among sections.

Ecological status as measured by mean values of the IPS appeared to vary seasonally: in spring IPS values ranged from 12.40 to 14.40, in summer fluctuated between 10.40 and 12.90 and

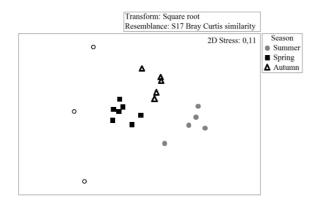


Figure 5. Two dimensional MDS plots based on Bray-Curtis similarities of square-root transformed diatom abundance data. Samples are ordered by seasons. *Gráfico Bidimensional MDS basado en similitudes Bray-Curtis de datos transformados a la raíz cuadrada de la abundancia de diatomeas. Las muestras se ordenan por estaciones del año.*

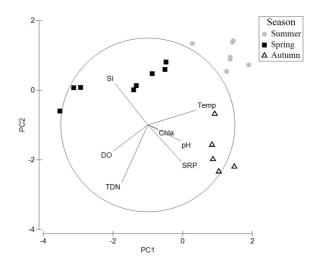


Figure 6. PCA of the diatom data showing seasonal ordination as a function of the environmental variables. Samples are ordered by seasons. *PCA de datos de diatomeas mostrando la ordenación estacional en función de las variables ambientales. Las muestras se ordenan por estaciones del año.*

during autumn from 8.70 to 13.70. The highest values occurred in E3 and would indicate "good" (spring and autumn) and "moderate" (summer) ecological status. The lowest values were found in E5, indicating "poor" (autumn) and "moderate" (spring and summer) ecological status.

DISCUSSION

This work includes a characterization of benthic communities (macroinvertebrates and diatoms) in a large Mediterranean river according to environmental variables and human pressures such as damming, covering wide aspects of the morphological variability of the lower Ebro River. The sampling of biological communities conducted in the littoral area, as it is often the case in the monitoring programs of the WFD in large rivers (including the lower Ebro River), given the technical complexity and economic cost of sampling the main river channel. At the beginning of the study the research team tried to obtain benthic samples in the channel with different types of drags but it was not possible because of high water velocity and type of dominant substrate (coarse gravel).

The annual average water temperature (17.30

°C) found at E5 was lower than the value found at E1-4 (19.59 °C). The seasonal water temperature variation was influenced by the presence of a nuclear power station located downstream E5 and by reservoirs located upstream. Their co-occurrence have opposite effects on the river water during summer and winter; this pattern has been previously documented by Prats et al. (2010) who noted that in summer the cooling effect of the reservoirs and the warming effect of the nuclear power station compensated each other; whereas in winter, the warming effect of both is added. The existence of thermal pollution in the lower Ebro River has given the opportunity to study the effect of water warming on macroinvertebrates and diatoms in the vicinity of the Ascó Nuclear Power Station (Quevedo, 2015). Dissolved oxygen followed the same tendency with mean values of 87.75 % and 94.67 % for E5 and E1-4 respectively, and minimum summer daily values at E5 dropping down to 30 %; this is due to the summer stratification of the reservoirs which release water from the hypolimnion with low oxygen and high nutrient content (Sabater et al., 2008).

Benthic macroinvertebrate communities at the study area showed some significant spatial and temporal differences. The multivariate analysis defined two different communities as a function of the distance to dams: the uppermost sampling section located close to the Flix dam (E5) was significantly different from all the other sections located downstream (E1-4); this could be related to the direct influence of the Riba-roja and Flix dams on the first section of the stretch causing changes in substrate composition (coarser substrate due to erosion of fine materials) and water quality (lower oxygen values and lower temperature), as well as higher chemical pollution (heavy metals, persistent organic compounds) due to the toxic waste accumulated in Flix reservoir. Previous studies on macroinvertebrates have shown higher bioaccumulation of heavy metals and pesticides in areas of the lower Ebro River closer to the Flix dam (Cid et al., 2010; Alcaraz et al., 2011). Thus, the present study cannot discriminate which part of the effect of reservoirs on benthic macroinvertebrates is due to hydromorphological impacts and to chemical pollution.

Results suggest that hydrogeomorphical

variability classified according to the four categories of the simplified Rosgen classification (see methods) was likely not a significant factor determining the structure of benthic communities in the lower Ebro River. As mentioned before, community structure was significantly different in the section closer to the dams, but also the ecological status measured with the WFD indicators (IBMWP and IPS), suggesting that this was mostly due to the impact of dams (changes in the hydrology, water temperature, oxygen nutrients, etc.) rather than to the particular hydromorphic category of this section. However, the presence of reservoirs affect the substrate composition of the river bed downstream (Batalla et al., 2004), with more dominance of coarser materials close to the dam, but this will happen in any of the defined hydromorphological categories. This outcome is in agreement with the conceptual framework of Serial Discontinuity (Ward & Stanford, 1983) proposed for regulated rivers, in which the distance to the dam is a determinant factor explaining differences in physical parameters and biological communities.

Changes in abundance and diversity of macroinvertebrate fauna as consequence of flow regulation has been previously reported worldwide; for instance Poff & Zimmerman (2010) in a review of 165 papers published over the last four decades found that macroinvertebrates showed mixed responses to changes in flow magnitude, with abundance and diversity both increasing and decreasing in response to elevated flows and to reduced flows. Similar reports are found in studies from large Mediterranean rivers (e.g. Bournaud et al., 1996; Chatzinikolaou et al., 2006; Marchetti et al., 2011; Muñoz & Prat, 1996) and around the world (Statzner *et al.*, 1988; Poff et al., 1997; Bonada et al., 2007; Tupinambás et al., 2014, 2015) pointing the regulation system as one of the main anthropogenic alterations on macroinvertebrate communities. In fact, environmental flows are now considered as a key issue to achieve the good ecological status of superficial water bodies as required by the Water Framework Directive of the European Union (European Commission, 2012).

Although the level of nutrients has been reduced during the last decades in the lower Ebro

River (Ibáñez et al., 2012), Chironomidae and Oligochaeta have a dominant status compared with groups as mayflies (Ephemeroptera) and caddisflies (Trichoptera). This pattern has been observed in streams and rivers with high levels of nutrients (Hawkes & Davies, 1971; Metcalfe, 1989; Whitehurst & Lindsey, 1990; Tupinambás et al., 2015) and, in the lower Ebro this could be also in part explained by the presence of toxic pollution in the Flix reservoir. The macroinvertebrate community along all the study area was characterized, in terms of relative abundance, by taxa that could be reflecting habitat degradation, such as Oligochaeta, Orthocladiinae, Echinogammarus, Baetis, Corbicula, Dugesia and Caenis. However, it is necessary a higher taxonomic identification to species level in order to reach an adequate ecological interpretation; for instance, Oligochaeta has been traditionally categorized as tolerant taxa, but many intolerant Oligochaeta species can be eradicated as consequence of pollution; this evidence that some Oligochaeta species are pollution tolerant but others not so much (Lafont, 2011). In fact, biotic indices based on Oligochaeta assemblages have been used in approaches for the refinement of biomonitoring programs (Lafont et al., 2010; Lafont et al., 2012).

The macroinvertebrate composition found in this study is remarkably different when compared to the community found in 1987 at E1 as reported by Muñoz & Prat (1994) and at E4 and E5 as reported by Limnos (1997) (Fig.2). Although the methodology used was somewhat different, it is possible to recognize relevant changes in the benthic community of the lower Ebro River. Among the main changes it can be highlighted the diversification of the community, the decrease of Trichoptera (especially *Hydropsyche exocellata*), Chironomidae and other taxa indicating eutrophication; and the arrival of invasive species such as Dreissena polymorpha and Corbicula fluminea. Historical changes in the density and production of Ephoron virgo, a filter feeding species that inhabits in sandy and fine gravel substrates, have been previously documented by Cid et al. (2008); it was abundant in the 80's (Ibáñez et al., 1991) and early 90's (Muñoz & Prat, 1994) but our results showed a decrease in abundance and distribution which could be associated to the

reduction in phytoplankton and the spread of the macrophyte pondweed Potamogeton pectinatus in the substrate occupied by this species. The decline of phytoplankton and the spread of macrophytes in the lower Ebro River at the beginning of the present century was mostly due to a large decrease in phosphorus, but the presence of invasive bivalves and the decline in river floods and suspended sediments may also play a role (Ibáñez et al., 2012). The first reports of the zebra mussel Dreissena polymorpha and the Asian clam Corbicula fluminea in the lower Ebro River date from 2001 and 1997, respectively; these species of bivalves (filter feeders) have quickly proliferated and may compete for food resources with other native filter-feeder species; in fact, during the study period specimens were found along all the sampling sections.

According to the statistical analysis, the composition of diatom assemblages was clearly influenced by seasonal variation, and this temporal variability is related to the fluctuating along-year conditions of the lower Ebro River which involves variation in sunlight intensity, changes in water temperature and differences in nutrient concentration, as well as changes in flow regulation. However, contrary to results obtained for macroinvertebrates, there were no significant differences among sections along the study area. This is consistent with the fact that macroinvertebrates and diatoms respond rather differently to anthropogenic stressors, being macroinvertebrates more sensitive to physical changes in river habitat, while diatoms are more sensitive to water quality alterations (Hering et al., 2006; Pace et al., 2012; Soininen & Könönen, 2004; Triest et al., 2001).

The most abundant diatom species in the study area (*Amphora pediculus*, *Cocconeis placentula* var. *lineata*, *Nitzschia dissipata*, *N. inconspicua* and *N. palea*) are also common further upstream in the Ebro River (http://www.chebro.es/contenido.visualizar.do?idContenido=27971&id-Menu=4101) as well as the fresher parts of its estuary (Rovira *et al.*, 2012a; Rovira *et al.*, 2012b), and more generally in many lowland rivers of Europe (e.g. Almeida & Feio, 2012; Urrea & Sabater, 2009; van Dam *et al.*, 2007). All the common species are widespread in α-,β-mesosaprobous waters (van Dam *et al.*, 1994).

Seasonal changes in diatom communities have also been found in rivers elsewhere (e.g. Gomà et al., 2005; Leira & Sabater, 2005; Martínez de Fabricius et al., 2003; Sherwood et al., 2000; Soininen & Eloranta, 2004). Changes are bound to occur in diatom communities during the year as a result of variation in light intensity, day-length, temperature and life cycles of grazers, but an extra factor in some Mediterranean rivers (e.g. Ebro, Po, Rhône) is the marked seasonal variation in flow as a result of snow-melt in spring and low summer precipitation (exacerbated by irrigation and industrial demand). Water flow variation could be a major factor controlling the seasonal changes observed in all analysed sections, consistent with the findings of Boix et al. (2010), Martínez de Fabricius et al. (2003) and Tang et al. (2013).

From the biomonitoring point of view it is interesting that all five sections had similar communities according to ANOSIM analysis and showed similar seasonal changes; this implies that all of them are representative of the whole stretch and therefore any section could be chosen for surveillance of the ecological status. However, the IPS scores were always lowest in section E5, the section closest to the regulation system, and at times indicated "poor" ecological status (in autumn) which is consistent with macroinvertebrate IBMWP scores, where again "poor" status was recorded only in E5 (although in spring). Otherwise, diatom (IPS) and macroinvertebrate (IBMWP) indices indicated "moderate" "good" ecological status.

The fact that in many cases the ecological status was "good" according to diatoms and macroinvertebrates does not mean that the overall ecological status, including other biological indicators, can be considered to be "good" as well. For instance, the fish community of the lower Ebro and other regulated rivers is strongly dominated by invasive species which are favored by dam regulation and river flow reduction (e.g. Bunn & Arthington, 2002; Gido *et al.*, 2013; Kiernan *et al.*, 2012; Lytle & Poff, 2004; Maceda-Veiga *et al.*, 2010; Olden *et al.*, 2006; Propst & Gido, 2004; Caiola *et al.*, 2014), and the ecological status of the lower Ebro River according to this indicator varies between "poor" and

"bad" (Sostoa et al., 2010). Furthermore, the absence of reference conditions in the lower Ebro regarding the biological communities before dam construction difficult the proper ecological quality assessment; however, even when reference conditions are well established, biological communities may also shift as consequence of factors as climate change.

No significant differences in community structure of diatoms and macroinvertebrates were found in sections E1 to E4 (and also in E5 for the case of diatoms), and the reason could be in part related to the methodology used for sampling, which did not cover all the internal variability of the river ecosystem since samples were collected in wadeable areas; thereby the littoral community is well represented, but we were not able to obtain information of the communities inhabiting the river channel due to the difficulty of sampling with high water flow and coarse substrate (trials with different types of dredges did not work). In addition, as Chironomidae and Oligochaeta were the dominant taxa in the macroinvertebrate community, higher taxonomic resolution for these groups may be necessary in future studies to be able to discriminate significant differences in community composition that could be relevant for a finer characterization of the ecological status. Specific methods based on biological indicators capable of integrating responses to different impacts could be complementary in order to achieve a more comprehensive assessment of the ecological status of large rivers subject to multi-stressor conditions.

CONCLUSIONS

The regulation system seems to be a main factor determining the structure of benthic communities in the lower Ebro River, but differences in benthic communities are only found in the section located close to the dams for the case of macroinvertebrates. While, the composition of the diatom assemblages was clearly influenced by the seasonal variation but not along the study area, according to the performed statistical analyses. However, the obtained scores of indices for ecological status assessment based on macroinvertebrates but also on diatoms, tended to be

lower in the section closest to reservoirs. It seems that these two groups responded rather differently to anthropogenic stressors; macroinvertebrates were more sensitive to physical and hydrological changes in river habitat, while diatoms were likely more sensitive to water quality alterations, but further studies need to be performed to better understand the changes in benthic communities under a multi-stressor scenario

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Con el apoyo de:





