

# ANALYZING THE EFFECTS OF STREAM-AQUIFER INTERACTIONS ON MACROINVERTEBRATE COMMUNITY STRUCTURE

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## Abstract

Mediterranean rivers are affected by natural disturbances and human pressures, which may alter their hydrology, water quality and habitat structure. In this study an analysis of macroinvertebrates community structure has been used to determine how distinct human pressures may affect these ecosystems. A total of 4 sampling campaigns were conducted (from November 2010 to July 2011) in the middle reach of the Onyar River (NE Spain), comprising stream discharge and physicochemical parameters measurements *in situ*, as well as water samples and macroinvertebrates assemblages, which were taken and properly preserved for subsequent analysis and identification. Results of variation partitioning showed that macroinvertebrate community structure was particularly dependent on hydrochemical characteristics, but also on hydrological variations. In addition, results also indicated that groundwater withdrawal altered stream hydrology, and the main reach of the Onyar River became intermittent and even completely dry in downstream positions. This reduction on stream discharge caused changes in habitat characteristics, as well as in the proportion of wastewater. Some wastewater dilution occurred, linked to groundwater with high nitrate concentrations inputs to the stream, and even though the macroinvertebrate community recovered its quality in some sampling campaigns, it presented a different structure than in sampling points not affected by any of these pressures. Therefore, the approach here used to analyze the hydrological effects on macroinvertebrate assemblages allowed us to determine their influence on hydrochemical and habitat characteristics.

**Keywords:** Surface water-groundwater interactions, hydrological alterations, groundwater exploitation, macroinvertebrate community

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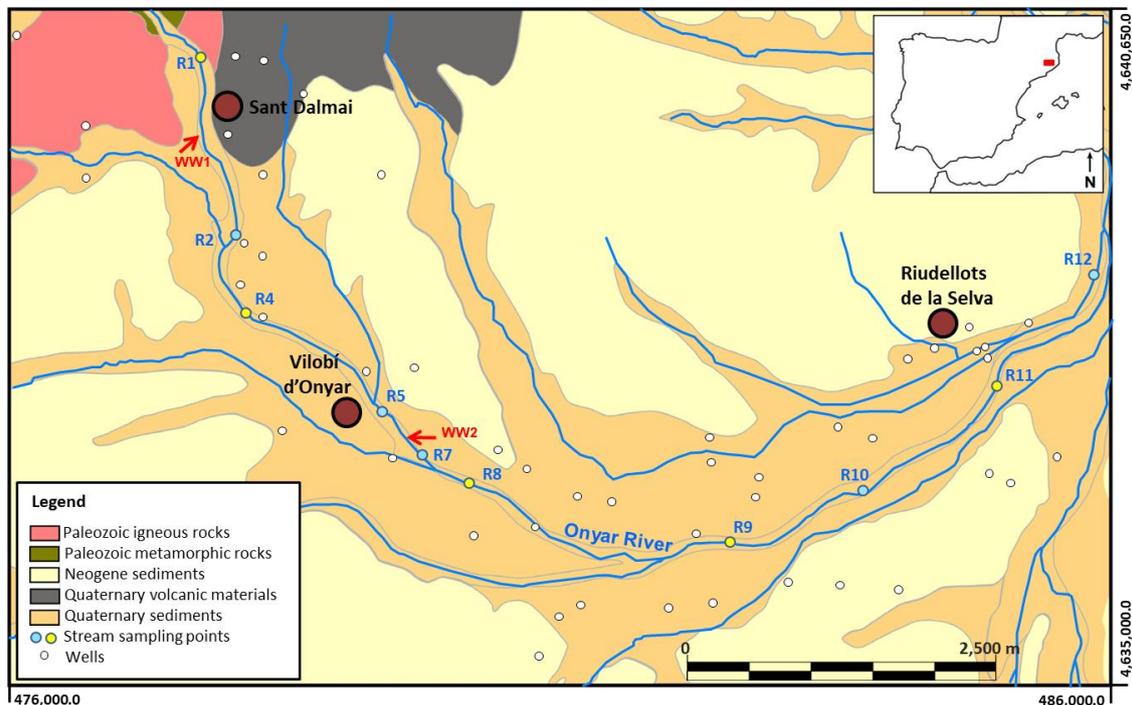
## 1. INTRODUCTION

Mediterranean streams are characterized by a variable flow regime with dry summers and winters, and rainy springs and autumns. These ecosystems are also affected by seasonal disturbances, such as floods and droughts (Bonada et al., 2007). However, streams may also be affected by human pressures, which may alter their hydrology, water quality and habitat structure. Alterations on flow regime caused by water regulation, direct extractions or groundwater overexploitation reduce streams availability to support a functional aquatic ecosystem (e.g., Benejam, et al., 2010; Belmar et al., 2013; Menció et al., 2014). Moreover, water quality can be modified by different types of pressures: urban and industrial wastewater discharges, whether treated or not, can be considered as a point type pressures in these systems; and streams may also be affected by non-point source pollution caused by surface and subsurface runoff from croplands and urban areas. As a result of these impacts on hydrochemistry, hydrology and habitat characteristics, changes in community population structure and even species survival were observed (e.g., Rubio-Gracia et al., 2017).

The aim of this study was to analyze how distinct human pressures (especially those linked to groundwater withdrawal) may affect macroinvertebrates community in the Onyar River (NE Spain; Figure 1). The Onyar River watershed has an area of 295 km<sup>2</sup> and is located in the Selva tectonic depression, about 100 km NE of Barcelona (NE Spain). The study area has a Mediterranean climate with a mean annual precipitation of  $695.9 \pm 168.7$  L/m<sup>2</sup> and a mean temperature of  $14.3 \pm 0.7$  °C (for the 1950-2011 period; Catalan Meteorological Service, <http://www.meteo.cat>). The Onyar River presents a mean flow of  $1.59 \pm 1.22$  m<sup>3</sup>/s, and a minimum monthly flow of 0.46 m<sup>3</sup>/s in summer (for the 1959-2011 period; Catalan Water Agency (ACA), <http://aca-web.gencat.cat/>). The alluvial deposits of the Onyar River are well developed along the main stream (downstream of R1 sampling point in Figure 1), showing a maximum thickness of 15 m approx., constituting a significant groundwater reservoir, and allowing effective base-flow control during dry seasons under no-exploitation conditions. In this watershed, several agricultural and urban uses modify stream flow, and surface

and groundwater quality. Intense groundwater abstraction for agricultural purposes causes severe head level downward, capturing the stream flow. Besides, treated wastewater effluents, and wastewater discharges also affect this stream. Finally, this area has been classified as a “Nitrate Vulnerable Zone”, and nitrate concentrations are higher than 50 mg/L in most of the boreholes.

Consequently, the specific objectives of this study were to determine the effects of hydrology on habitats and stream water quality in order to assess the role of hydrology on macroinvertebrate assemblages in anthropized environments.



**Figure 1.** Geographical and geological situation of the study area, and sampling points' location. Legend: WW, wastewater discharge; stream sampling points with blue infilling are those where only hydrochemical and discharge data were measured, while the ones with yellow infilling, are those with macroinvertebrate assemblages.

## 2. METHODS

A total of 4 sampling campaigns (from November 2010 to July 2011) were conducted in the middle reach of the Onyar River (with a total length of 11 km in this area), using ten sampling points located at variable distances in the main reach. Stream discharge and physicochemical parameters, such as temperature, Electrical Conductivity (EC), pH, Eh, and Dissolved Oxygen (DO) were measured *in situ*. Stream water samples and macroinvertebrate assemblages were taken properly in these sites and preserved for subsequent analysis and identification. Hydrochemical analysis determined Alkalinity as  $\text{HCO}_3^-$ ,  $\text{Cl}^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{SO}_4^{2-}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{Br}^+$ ,  $\text{K}^+$ , and nitrogen as total Kjeldahl nitrogen (TKN),  $\text{NH}_4^+$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$ . All analyses were conducted according to APHA (2005) and their quality was checked performing the ionic mass balance, with all samples showing an error lower than 5%.

Macroinvertebrate taxa were identified at genus level under a Motic SMZ-168-BL zoom stereo microscope (50x magnification), except for Diptera and Platyhelminthes that were identified at family level and Oligochaeta that were identified at subclass level. These assemblages were taken in R1, R4, R8, R9 and R11 sampling points.

The comparison of macroinvertebrate assemblages between sampling points, seasons and the influence of wastewater or groundwater in the stream discharge, was performed by means of Analysis of Similarities (ANOSIM, Clarke & Warwick, 2001). Additionally, non-parametric multidimensional scaling (MDS) was performed in the Onyar River samples to analyze the relationship between macroinvertebrate structure with respect to their location along the stream reach analyzed. Both analyses were performed by PRIMER v.6.0 for Windows.

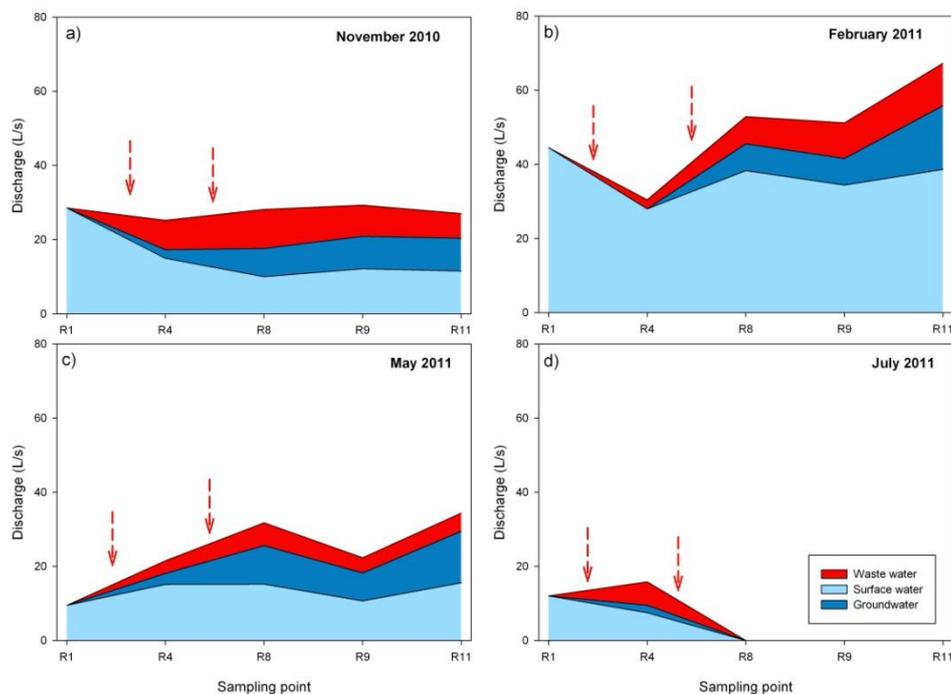
Therefore, the effects of hydrological variables, hydrochemical conditions and habitat characteristics on the different biotic components were analyzed by means of variation partitioning (Bocard et al., 1992;

Legendre & Legendre, 1998) using the “vegan” package (Oksanen et al., 2009) of R language (R Development Core Team, 2008). Variation partitioning was here used to decompose the variation of dependent variables in unique (or pure) and shared (or joint) effects of a set of predictors. The following indicators and sets of variables were considered for this analysis: a) three different variables were taken into account to consider hydrologic characteristics (the proportion of surface water, groundwater and waste water in L/s and also in % determined in each sampling point through End Member Mixing Analyses (EMMA), and also the Incremental Stream Flow Measure based on stream discharge measures; Menció et al., 2014); b) habitat characteristics were determined using the percentage of the 12 substrata types defined in the previous section, as well as the Fluvial Habitat Index (IHF index) for Mediterranean streams described by Pardo et al. (2002); and c) hydrochemical parameters, as well as the results of a Principal Component Analysis (PCA) considering all the hydrochemical parameters. Finally, a matrix based on macroinvertebrate data was created, containing the Hellinger transformation (square-root transformation) of the abundances individuals of each assemblage according to the taxonomic level determined, in order to express the data as relative abundances (Bocard et al., 2011).

### 3. RESULTS

#### 3.1. Sampling sites characteristics

Ten sampling points were selected, at variable distances, to track discharge changes related to stream-aquifer exchange or waste water treatment plant inputs along the Onyar River (R1, R2 R4, R5, R7 R8, R9, R10, R11 and R12 in Figure 1; based on Menció et al., 2014). In Figure 2 the distribution of the different sources of stream discharge, classified as surface water, groundwater and wastewater, has been represented for all sampling campaigns in those locations where macroinvertebrate assemblages were taken (R1, R4, R8, R9 and R11).



**Figure 2.** Dynamics of discharge and sources (in L/s), according to Menció et al. (2014), for: a) November 2010, b) February 2011, c) May 2011, and d) July 2011 sampling campaigns. Legend: red dashed arrows, waste water discharge points; in July campaign, R8 and R9 were intermittent and R11 was completely dry.

The Onyar River was not a permanent stream due to the capture of its flow during the summer season (Figure 2d). This situation was caused by groundwater withdrawal for agricultural purposes, and the continuous flow in the main stream was not recovered until significant precipitations occur in autumn. Therefore, during summer season the stream was intermittent from R8 to R9, and completely dry in R11 (Figure 2d).

Upstream R1 sampling site there was not, nor an extensive alluvial aquifer, neither any waste water discharge. For this reason, the only source of water considered in R1 was surface water. Downstream, the

studied reach of the Onyar River received two continuous wastewater discharges before sampling points R4 and R8 (Figure 2), and thus, the proportion of wastewater in the stream flow increased. Finally, the proportion of groundwater contribution to the stream flow varied depending on the sampling campaign.

As regards habitat characteristics, differences were observed for IHF values between sampling points ( $F=7.353$ ,  $df=4$ ,  $p=0.002$ ), and also for sampling campaigns. These differences were significant due to the distinct values of R1 with respect to the rest of the sampling points, and for the lower values of IHF in winter and summer, than in autumn and spring campaigns ( $t=3.845$ ,  $df=3$ ,  $p<0.001$ ;  $t=16.743$ ,  $df=4$ ,  $p=0.031$ ). The lowest values during winter were caused by a flooding event; and in summer, due to the loss of stream discharge caused by groundwater withdrawal (Figure 2).

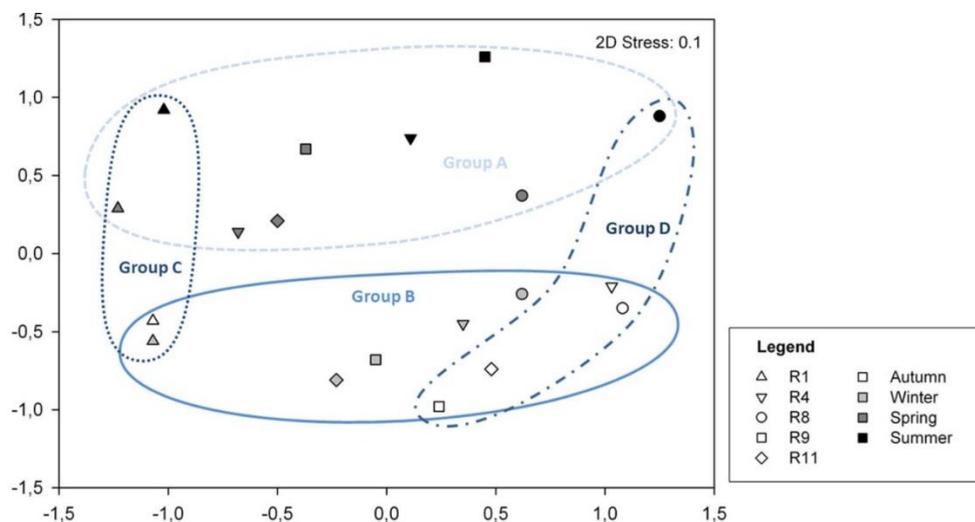
### 3.2. Assemblage variation results

Results of ANOSIM showed differences in assemblage composition among sampling points, between R1-R8 ( $R=0.999$ ,  $p=0.029$ ), R1-R11 ( $R=0.667$ ,  $p=0.029$ ) and R8-R11 ( $R=0.537$ ,  $p=0.029$ ). In contrast, R4 and R9 did not present significant differences to the other sampling points, showing shared assemblage compositions.

When assemblage compositions were compared among sampling campaigns, significant differences were obtained ( $R=0.417$ ,  $p=0.002$ ), showing two distinct groups of samples: one composed of spring and summer samples ( $R=0.319$ ,  $p=0.063$ ), and the other one, autumn and winter samples ( $R<0.01$ ,  $p=0.579$ ). When groundwater influence was tested based on the use of Incremental Stream Flow measures, three groups of samples were considered: L, sampling points with a losing stream situation; G, with a gaining stream situation; and NR, with non-relationship with the aquifer. Significant differences between the three groups of sampling points considered were obtained ( $R=0.213$ ,  $p=0.029$ ). These differences were significant for NR and L groups ( $R=0.405$ ,  $p=0.018$ ), and also between NR and G groups ( $R=0.405$ ,  $p=0.013$ ). A second classification was done considering two groups of samples based on EMMA results, and taking as a threshold for groundwater influence a 20% of groundwater influence. In this case, however, differences in assemblage compositions between the two groups of samples were not significant ( $R=0.08$ ,  $p=0.155$ ).

Finally, when wastewater effects were evaluated, two distinct classifications were taken into account: the first one, considering as a threshold a 20% of wastewater composition in stream discharge, and the second one, considering only a 15% (based on EMMA results). Considering the threshold of 20%, significant differences between the two groups of samples were obtained ( $R=0.207$ ,  $p=0.029$ ), but in the second case ( $>15\%$ ), these differences were not significant ( $R=0.107$ ,  $p=0.110$ ).

Non-parametric multidimensional scaling (MDS) plot was in accordance to ANOSIM results, showing differences between autumn and winter samples (group A) in contrast to spring and summer samples (group B in Figure 3). Additionally, two other groups could be distinguished: group C, composed of R1 site samples, and group D containing samples with a wastewater percentage higher than 20%.



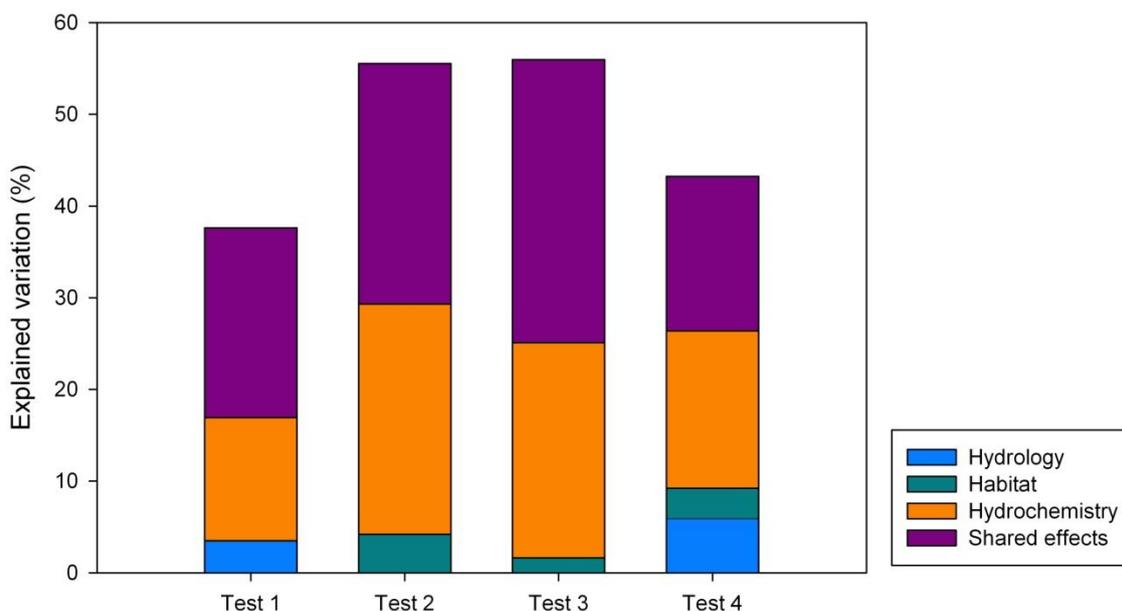
**Figure 3.** MDS plot of the samples of macroinvertebrate assemblages, according to sampling point and sampling campaign. Legend: Group A, spring and summer samples; Group B, autumn and winter samples; Group C, R1 samples; and Group D, most of the samples with  $>20\%$  of wastewater.

Finally, variation partitioning was conducted considering different sets of variables that were previously tested and selected to avoid collinearity (Table 1). In the case of hydrological variables two sets of data were used: one including the values of surface water, groundwater and wastewater in the stream as L/s (HDR1 in Table 1); and a second one using these data as percentage (HDR2). For habitat characteristics, the IHF index was used (Table 1). And finally, for hydrochemical data two distinct groups of variables were also used: a first one included the results of a Principal Component Analysis conducted with hydrochemical data, that is VF1 and VF3 (HCH1 in Table 1); and a second one, EC, T,  $\text{HCO}_3^-$ ,  $\text{Cl}^-$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Br}^-$ ,  $\text{NO}_3^-$  and  $\text{NH}_4^+$  (HCH2). VF1 was composed of those variables representing the influence of waste water effluents ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{HCO}_3^-$ ,  $\text{Mg}^{2+}$  and EC), while VF3 was linked to nutrients ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ) and temperature.

**Table 1.** Variables considered for variation partitioning. Legend: MACROH, matrix of transformed macroinvertebrate abundances using Hellinger distance; HAB, habitat variables; HCH, hydrochemical variables; HDR, hydrological variables; WW, wastewater; GW, groundwater; and SW, surface water.

Variable	Test			
	T1	T2	T3	T4
<b>Y: MACROH</b>	<b>MACROH</b> Macroinv. Data	<b>MACROH</b> Macroinv. Data	<b>MACROH</b> Macroinv. Data	<b>MACROH</b> Macroinv. Data
<b>X1: HDR</b>	<b>HDR1</b> WW, GW, SW L/s	<b>HDR1</b> WW, GW, SW L/s	<b>HDR2</b> WW, GW, SW %	<b>HDR2</b> WW, GW, SW %
<b>X2: HAB</b>	<b>HAB</b> IHF	<b>HAB</b> IHF	<b>HAB</b> IHF	<b>HAB</b> IHF
<b>X3: HCH</b>	<b>HCH1</b> VF1, VF3	<b>HCH2</b> EC, T, $\text{HCO}_3^-$ , $\text{Cl}^-$ , $\text{Na}^+$ , $\text{K}^+$ , $\text{Mg}^{2+}$ , $\text{Br}^-$ , $\text{NO}_3^-$ , $\text{NH}_4^+$	<b>HCH2</b> EC, T, $\text{HCO}_3^-$ , $\text{Cl}^-$ , $\text{Na}^+$ , $\text{K}^+$ , $\text{Mg}^{2+}$ , $\text{Br}^-$ , $\text{NO}_3^-$ , $\text{NH}_4^+$	<b>HCH1</b> VF1, VF3

Results obtained considering the different sets of variables (T1, T2, T3 and T4 in Table 1) were similar in all the analyses conducted (Figure 4). The total variation of the organism matrix explained by the descriptive variables ranged from 37.6-55.9%. The organism matrix was significantly related to the overall hydrochemical effects (with values ranging from 33-50% of variation), and also to the pure hydrochemical effects when HCH1 set of variables was considered (with values of 13 and 17% in T1 and T4, respectively). Hydrological data were only significant as overall effects (with values ranging from 23-30%), but not as pure effects. And finally, habitat effects were not significant, nor as overall effects, neither as pure effects (showing values <7% in all the analysis conducted; Figure 4).



**Figure 4.** Results of the four different variation partitioning tests conducted (variables considered are described in Table 1), with the total variation, pure and shared effects of the different sets of explanatory variables represented.

## 4. DISCUSSION AND CONCLUSIONS

Some distinct human pressures have been detected in the studied reach of the Onyar River. Groundwater withdrawal for agricultural purposes during late spring and summer altered stream hydrology, and the main reach of the Onyar River became intermittent in some sampling points (R8 and R9), and even completely dry downstream (R11 in Figure 2d). This decrease in stream discharge caused, on the one hand, changes in habitat characteristics that were depicted by the low IHF index values observed in summer. On the other hand, an increase up to 57% of wastewater proportion in stream discharge during summer and autumn surveys was caused by this reduction of stream flow, even though the dilution effect of groundwater contributions (particularly from R8 to R11; Figure 2a and d). In this situation hydrochemical deterioration of stream water, could be caused by two distinct sources of pollution: treated and non-treated waste water effluents (red arrows in Figure 2); and  $\text{NO}_3^-$  polluted groundwater discharge to the stream as baseflow (Menció and Mas-Pla, 2008).

When the effects of all the different variables (hydrology, habitat and hydrochemistry) on the variability of macroinvertebrate assemblage distribution were analyzed together through ANOSIM, non-parametric multidimensional scaling, and variation partitioning analysis results show that (Table 1 and Figure 3):

- a) habitat characteristics were not significant in any of the distinct tests conducted as pure or overall effects, despite the significant differences observed between sampling points and campaigns;
- b) hydrology was only significant as overall effects. ANOSIM results also showed that groundwater influence differences were significant when sampling points were classified according to their relationship with the stream;
- c) hydrochemical parameters, which were mainly related to wastewater and nitrate pollution, were significant as both pure effects and overall effects. Besides, when a threshold of 20% of wastewater composition was considered, significant differences were also obtained with ANOSIM.

These results were in accordance to the hydrological and hydrochemical situation of this stream. Macroinvertebrate assemblages may be affected by the hydrochemical characteristics of the sampling point; however, these characteristics were linked to the hydrological dynamics, since depending on groundwater contribution to the stream flow, the stream presented different hydrochemical qualities. The effects of flow regime alterations on macroinvertebrates have been widely analyzed. Under natural conditions, some authors have reported some minor differences in faunal composition between perennial and temporary rivers and an overlap between macroinvertebrate assemblages (Boulton & Lake, 1992; del Rosario & Resh, 2000; Bonada et al., 2007); in contrast, others have found significant differences (Legier and Talin, 1973; Boulton et al., 2000). Furthermore, flow regime alterations caused by human activities, such as water regulation, have been reported by several authors showing their negative effects on macroinvertebrate communities (Sharma et al., 2005; Boix et al., 2010; Theodoropoulos & Iliopoulou-Georgudaki, 2015). In particular, flow regime alterations caused by groundwater extraction and its effects on macroinvertebrate assemblages have been addressed in some recent studies showing its negative effects in some cases (e.g. Kløve et al., 2011; Bradley et al., 2017). In this study, however, flow regime alterations caused by groundwater abstraction in the Onyar River area have been reported, together with hydrochemical quality degradation.

In conclusion, the approach here used to analyze the hydrological effects on macroinvertebrate communities allowed us to determine their influence on hydrochemical and habitat characteristics.

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