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Parent lithology and organic matter influence the hyporheic biota of two Mediterranean rivers in central Spain

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ABSTRACT

Parent lithology and organic matter influence the hyporheic biota of two Mediterranean rivers in central Spain

The hyporheic zone of stream ecosystems, located at the transition between surface channel and ground waters, exhibits patchy discontinuity on a longitudinal transect and induces changes in the structure and functioning of biotic communities. Nevertheless, the factors that influence biotic communities along spatial and temporal gradients remain poorly understood. This study aimed to characterise the diversity and spatial pattern distribution of Cyclopoida and Ostracoda from the hyporheic zone in relation to the riverbed sediment structure and the quantity of organic matter in two second-order rivers in central Spain, whose alluvium is derived from materials of contrasting geology (siliceous vs. carbonates). Both streams were found to be characterised by marked habitat heterogeneity along the hyporheic flow path, as reflected by the different lithology, riverbed permeability and distinct hyporheic biota assemblages. The results indicate that the alluvium riverbeds dominated by carbonates rocks and associated with high permeable riverbeds had slightly diverse hyporheic assemblages (25 species in all) with species of mixed ecology (stygobites/stygophiles/stygoxenes). Conversely, the siliceous alluvium riverbeds with low mineralised waters, associated with reduced permeability and relatively less active surface/ground water exchanges, displayed slightly lower diversity (23 species) with assemblages exclusively composed of stygophiles/stygoxenes. The results highlight the role of the riverbed substratum for the hyporheic biota and provide forthcoming approaches for depicting surface-subsurface hydrological exchanges.

Key words: Hyporheic zone, diversity, organic matter content, sediment structure.

RESUMEN

Influencia de la litología y la materia orgánica sobre la biota hiporreica de dos ríos Mediterráneos en la zona centro de España

La zona hiporreica de los ecosistemas fluviales, situada entre las aguas superficiales y subterráneas, posee una gran heterogeneidad a lo largo de su perfil longitudinal que provoca cambios en la estructura y funcionamiento de las comunidades bióticas. Sin embargo, los factores que afectan a las comunidades bióticas a lo largo de un gradiente espacial y temporal son aún poco conocidos. En este estudio se pretende caracterizar la diversidad y el patrón de distribución espacial de los ciclopoides y ostrácodos de la zona hiporreica, de acuerdo a la estructura y cantidad de la materia orgánica contenida en el sedimento derivado de materiales geológicos diferentes (materiales carbonatados frente a silíceos) pertenecientes a dos ríos de segundo orden en el centro de España. Ambos ríos se caracterizaron por presentar una gran heterogeneidad de hábitats hiporreicos a lo largo de su perfil longitudinal, tal y como se refleja en cambios en la litología, la permeabilidad de los materiales que componen el cauce del río y las distintas comunidades bióticas asociadas. Los resultados indican que los cauces de los ríos dominados por carbonatos y con materiales de alta permeabilidad presentaron unas comunidades faunísticas levemente más diversas (25 especies en total) y con una ecología mixta (estigobiontes/estigofilas/estigoxenas). Por el contrario, los cauces de los ríos dominados por materiales silíceos con aguas poco mineralizadas y pocos intercambios entre agua superficial/agua subterránea, debido a una menor permeabilidad de los materiales, mostraron una menor diversidad (23 especies) con comunidades principalmente formadas por especies estigofilas y estigoxenas. Los resultados obtenidos ponen de manifiesto la importancia que tiene el origen de los materiales que conforman los sedimentos hiporreicos *en la composición y distribución de las comunidades hiporreicas, así como para la determinación de procesos de intercambios hidrológicos entre las aguas superficiales y subterráneas.*

Palabras clave: *Zona hiporreica, diversidad, contenido de materia orgánica, granulometría.*

INTRODUCTION

The hyporheic zone (HZ) is a band of saturated permeable sediments that lies adjacent to the channel of a river/stream where surface and ground waters mix (sensu Triska *et al*., 1989; Orghidan, 2010). The HZ is a highly dynamic ecotone. On a microscale, it is highly heterogeneous and results from the riverbed mosaic structure and surface-groundwater interactions (White *et al*., 1987; White, 1993; Dole-Olivier *et al*., 2009; Dole-Olivier, 2011). The primary production in the HZ depends on allochthonous inputs of organic matter: dead plants, organisms, microbes and products of their metabolism (Barrera-González *et al*., 2014). Organic matter fuels hyporheic biota, acts as a food resource and maintains trophic chains.

The origin of organic matter in the HZ varies from direct introduction into the stream from riparian vegetation (leaf litters and woods) to downwelling water or being buried during sediment transport events (Brunke & Gonser, 1997; Franken *et al*., 2001; Elosegui & Pozo, 2005; Findlay, 2006; Wallace *et al.,* 2006; Cornut *et al*., 2012; Tonin *et al*., 2014). Leaf litters are further fragmented and transformed into fine particulate organic matter or dissolved organic matter by physical (i.e., turbulence and water flow) and biological (i.e., microbial activity and organism's breakdown) processes (Wallace *et al*., 2006; Pozo *et al*., 2009; Tonin *et al*., 2014). The second source of organic matter in the HZ is upwelling groundwater and surrounding floodplain soil washes (Franken *et al*., 2001; Tione *et al*., 2011; Trimmer *et al*., 2012).

A particular feature of HZ is its capacity to load organic matter along the riverbed, which is influenced mainly by the HZ size, the structural pattern of coarse gravel riverbeds and spatial segregation in the bed form distribution (Jones, 1997; Pozo *et al*., 2009; Cornut *et al*., 2012). Organic matter appears in the HZ as a solute on hyporheic waters and as decaying organic particles, dissolved molecules, free-living organisms and attached biofilms in sediments, and is further retrieved, transformed and mobilised by heterotrophic living organisms (Boulton *et al*., 1998). Organic matter is significantly concentrated in the upper sediment layers of downwelling zones, and its distribution along the HZ profile is patchy and influenced by the input rate, abiotic and biotic processing, sediment structure, grain size distribution and flood events, which imply bedload movements (Findlay *et al*., 1993; Brunke & Gonser, 1997; Franken *et al*., 2001).

Early studies on the distribution of organic matter among sediments have indicated that it strongly influences the distribution of hyporheic meiofauna, as reflected in the heterogeneity of the community's diversity, abundance and distribution (Ward *et al*., 1994; Roca & Wansard, 1997; Baker *et al*., 2000; Franken *et al*., 2001; Fowler & Scarsbrook, 2002; Tione *et al*., 2011; Ruíz *et al*., 2013). Although several research studies have analysed the influence of hydrological and hydrochemistry characteristics on hyporheic biota community structures (Danielopol, 1989; Boulton *et al*., 1998; Gibert *et al*., 1994; Di Lorenzo *et al*., 2013; Iepure *et al*., 2013, 2014), the influence of substrate heterogeneity is poorly understood (Brunke & Gonser, 1997; Franken *et al*., 2001; Dole-Oliver, 2011).

This paper aims to describe the effect of the small-scale distribution of HZ cyclopoid and ostracod crustaceans in relation to: i) heterogeneity in the spatial and temporal distribution of organic matter (in water and sediments); and ii) the substrate riverbed structure (particle size and lithology) in two small Mediterranean streams in central Spain with contrasting geological compositions. Our central hypothesis is that the exis-

Table 1. Sampling site information. Key: H(1-9) = Henares sampling sites; T(1-11) = Tajuña sampling sites; WWTP = wastewater treatment plant discharges; Sil. = siliceous materials; Carb. = carbonate materials; Sulph. = sulphate materials (gypsum). *Información de los puntos de muestreo. Clave: H(1-9) = puntos de muestreo en el Río Henares; T(1-11) = Puntos de muestreo en el Río Tajuña; WWTP = vertidos de estaciones depuradoras de aguas residuales; Sil. = materiales siliceos; Carb. = materiales carbonatados; Sulph. = materiales sulfatados (yesos).*

Parameter Site	Location	Coordinates UTM		Elevation	River sector	Distance to headwaters	Lithology	Main disturbances
		x	*y*	(masl)		(km)		
Henares								
H1	Sigüenza	536 743	4 551 802	1033	Upper	2.7	Carb.	Sewage waters, agriculture
H2	Jadraque	506 873	4 533 371	802	Upper	37.5	Carb.	Sewage waters, agriculture
H3	Heras de Ayuso	489 211	4 518 039	691	Middle	61.4	Sil./Carb.	Gravel pit site
H4	Fontanar	486 203	4 507 677	651	Middle	70.3	Sil./Carb.	WWTP
H5	Guadalajara	483 552	4 496 957	642	Middle	75.6	Carb.	WWTP
H6	Alovera	480 141	4 489 757	620	Lower	86.2	Carb./Sulph.	WWTP
H7	Los Santos de la Humosa	476 254	4 486 630	601	Lower	92.3	Carb./Sulph.	WWTP
H8	Alcalá de Henares	463 706	4 478 084	575	Lower	106.9	Carb./Sulph.	WWTP
H9	Mejorada del Campo	458 514	4 473 721	551	Lower	114.7	Carb./Sulph.	WWTP
Tajuña								
T1	Luzón	560 737	4 542 203	1177	Upper	6.4	Carb.	Sewage waters, agriculture
T2	Peña Horadada (Luzón)	556 615	4 543 428	1165	Upper	10.3	Carb.	Agriculture
T3	Luzaga	546 923	4 536 508	1069	Upper	21.4	Carb.	Sewage waters, agriculture
T4	Abanades	543 314	4 527 080	1040	Upper	29.2	Carb.	Sewage waters, agriculture
T5	Masegoso de Tajuña	525 538	4 518 993	875	Middle	48.4	Carb.	Dam, sewage waters, agriculture
T6	Romancos	508 237	4 505 244	783	Middle	70.2	Carb./Sulph.	Sewage waters, agriculture
T7	Armuña de Tajuña	497 350	4 486 827	703	Middle	90.1	Carb./Sulph.	Sewage waters, agriculture
T8	Loranca de Tajuña	491 890	4 478 334	668	Middle	99.9	Carb./Sulph.	Sewage waters, agriculture
T9	Orusco	482 575	4 458 990	612	Lower	119.8	Carb./Sulph.	WWTP, agriculture
T10	Chinchón	456 770	4 445 061	517	Lower	148.1	Carb./Sulph.	Dam, WWTP, agriculture
T11	Titulcia	451 320	4 442 448	501	Lower	153.9	Carb./Sulph.	Agriculture

tence of a longitudinal gradient along a stream profile in the organic matter content distribution, local riverbed substrate composition and grain size results in a continuous adjustment in the diversity and density of hyporheic biota.

METHODS

Study area

Our study was conducted in two second-order streams (Henares and Tajuña) in central Spain that flow through the provinces of Guadalajara and Madrid (Fig. 1). Both streams are developed in Quaternary alluvium riverbeds, which are 1-1.5 m in depth and at least 10 m in width, whose substrates are composed principally of pebbles, gravel, sand and silt (Arribas *et al.*, 2000; Camargo, 2006). The study streams were chosen for their contrasting riverbed structures, which comprise sediments derived from materials of distinct lithology: siliceous (Henares) *vs*. carbonates (Tajuña) (see Iepure *et al.*, 2014).

The Henares (H) stream is 158 km long with a catchment area of 4136 km^2 and a mean annual discharge of 3.30 m^3/s. The water discharge measured during the surveyed period in 2012 was 3.24 m^3/s in February and 4.92 m^3/s in May (SAIH-Tajo, 2014). On the longitudinal transect, distinct channel types are recognized (cf. Montgomery & Buffington, 1998): in the upper part, the channel is embedded in carbonate bedrocks with substrates of very high permeability (H1-H2); in the middle sector (between H3 and H5), the channel bedrock overlays a mixture of carbonates and siliceous rocks; the riverbed substrates of the lower sector (between H6 to H9) develop in a mixture of carbonates and gypsum-sulphates (Camargo, 2006; Garrote *et al.*, 2008).

The Tajuña (T) stream is 254 km long with a catchment area of 2607 km^2 and a mean annual discharge is 2.37 m^3/s. The water discharge measured during the surveyed period in 2012 was 2.63 m^3/s in February and 2.75 m^3/s in May (SAIH Tajo, 2014). The Tajuña stream channel develops fully on carbonated rocks (from T1 to T4) or on a mixture of carbonated rocks with gypsum (downstream T7) that exhibit high and very high permeability (Garrote *et al.*, 2008). The stream sector between T4 and T5 is dammed (La Tajera Lake, 450 ha, maximum depth > 10 m) and is fed by Tajuña streamwater, run-off and precipitation. The dam is used for agriculture by regulating the Tajuña stream water level in the summer and alters the discharge to the main downstream channel (Castañeda-Buendia, 2011).

Sampling design and methods

The HZ of both studied streams was investigated at 20 sampling sites distributed along their entire courses after selecting nine sites in Henares (H1-9) and 11 in Tajuña (T1-11) (Fig. 1; Table 1). Surveys were conducted in two temporal replicates in winter (February) and spring (May) of 2012. All of the sites had a permanent surface flow; only one site was frozen in winter (T2). At each sampling point, the geographical coordinates and elevation were determined using a Garmin GPS-76.

Samples of interstitial water, sediments and organisms were retrieved from two replicates located in the middle of the channel at a depth of 20-40 cm by pumping 12 L of material with a Bou-Rouch device (Bou & Rouch, 1967) and subsequently filtering this volume through a planktonic net (63 μm). Any retained biological and sediment samples were fixed in 70% ethanol until laboratory processing. The biological samples were stained with Eosin Yellowish after fixation to aid the separation of the specimens from sediments and detritus.

During each survey period, channel and hyporheic waters were analysed *in situ* to determine their temperature (Hanna, checktemp1), dissolved oxygen (Crison, OXI-45P), pH (Crison, pH-25) and electrical conductance (Crison, CM-35). One litre of water from both the stream channel and the HZ were collected and filtered in the laboratory through a glass-fibre filter (0.45 μm) to separate the dissolved organic carbon (DOC) fraction for the later analyses of 23 physico-chemical parameters. For the present study, the following nine chemical parameters were retained (in mg/l): biological oxygen demand (BOD_5), chemical oxygen demand (COD), non-purgeable organic carbon (NPOC), total organic carbon (TOC), total carbon (TC), inorganic carbon (IC), carbonates (CO_3^{2-}), bicarbonates (HCO_3^-) and sulphates (SO_4^{2-}) (see Supplementary information Table S1, available at www.limnetica.com).

The hyporheic organisms were retrieved from the sediments under an Olympus SZX-7 stereomicroscope and were counted and classified into classes and orders. Ostracods and cyclopoids were identified to the species level (Meisch, 2000; Dussart & Defaye, 2001) and

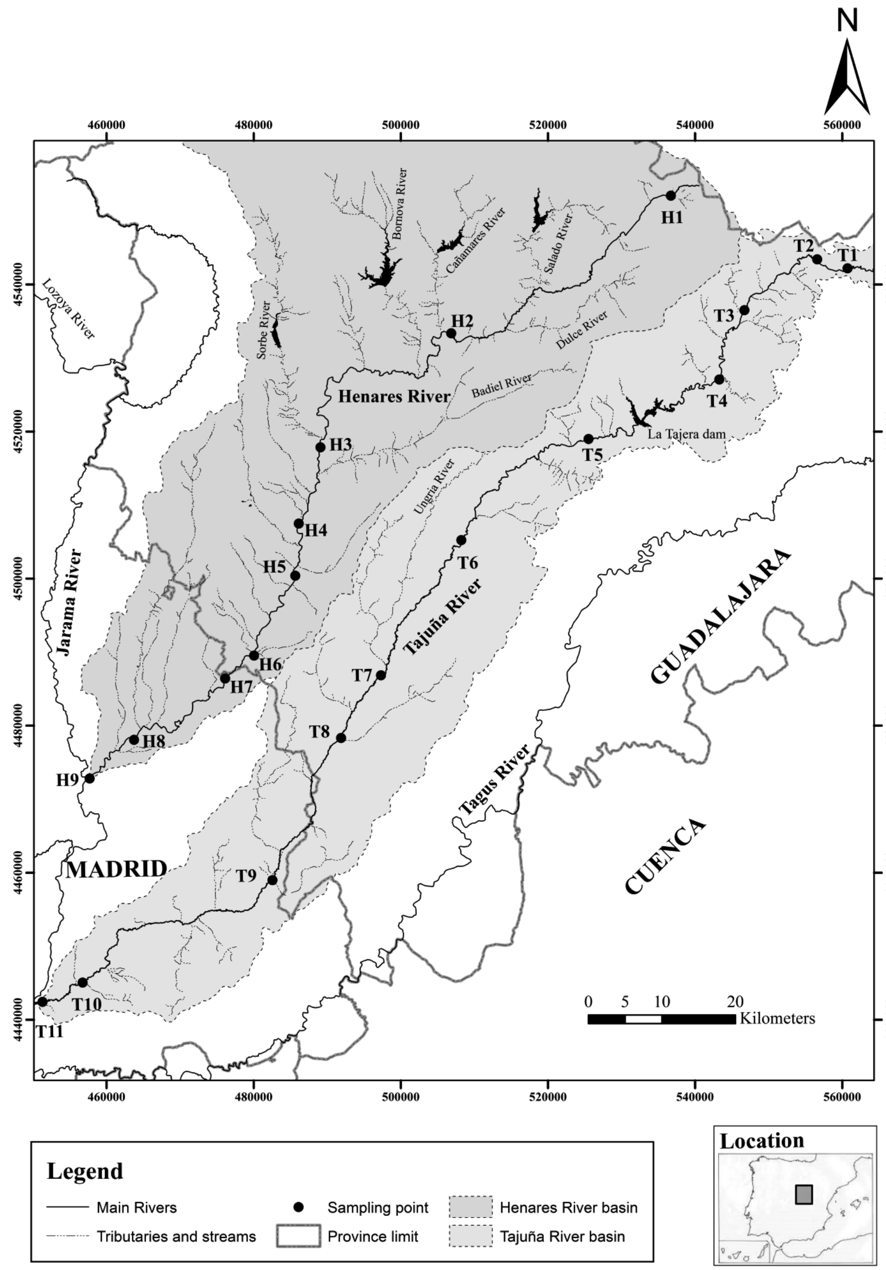

Figure 1. Location of the study area and distribution of sampling sites. *Localización del área de estudio y distribución de los puntos de muestreo.*

classified into the following ecological categories: stygobites and non-stygobites (*sensu* Gibert *et al*., 1994).

After removing the organisms, the interstitial sediments were retained for granulometric analyses. The sediments were primarily dried in a P-Selecta oven at 50 °C for 24-48 h. The substrates of each site were classified into five classes according to grain size: class 1, very coarse sand (Ø > 1 mm); class 2, coarse sand

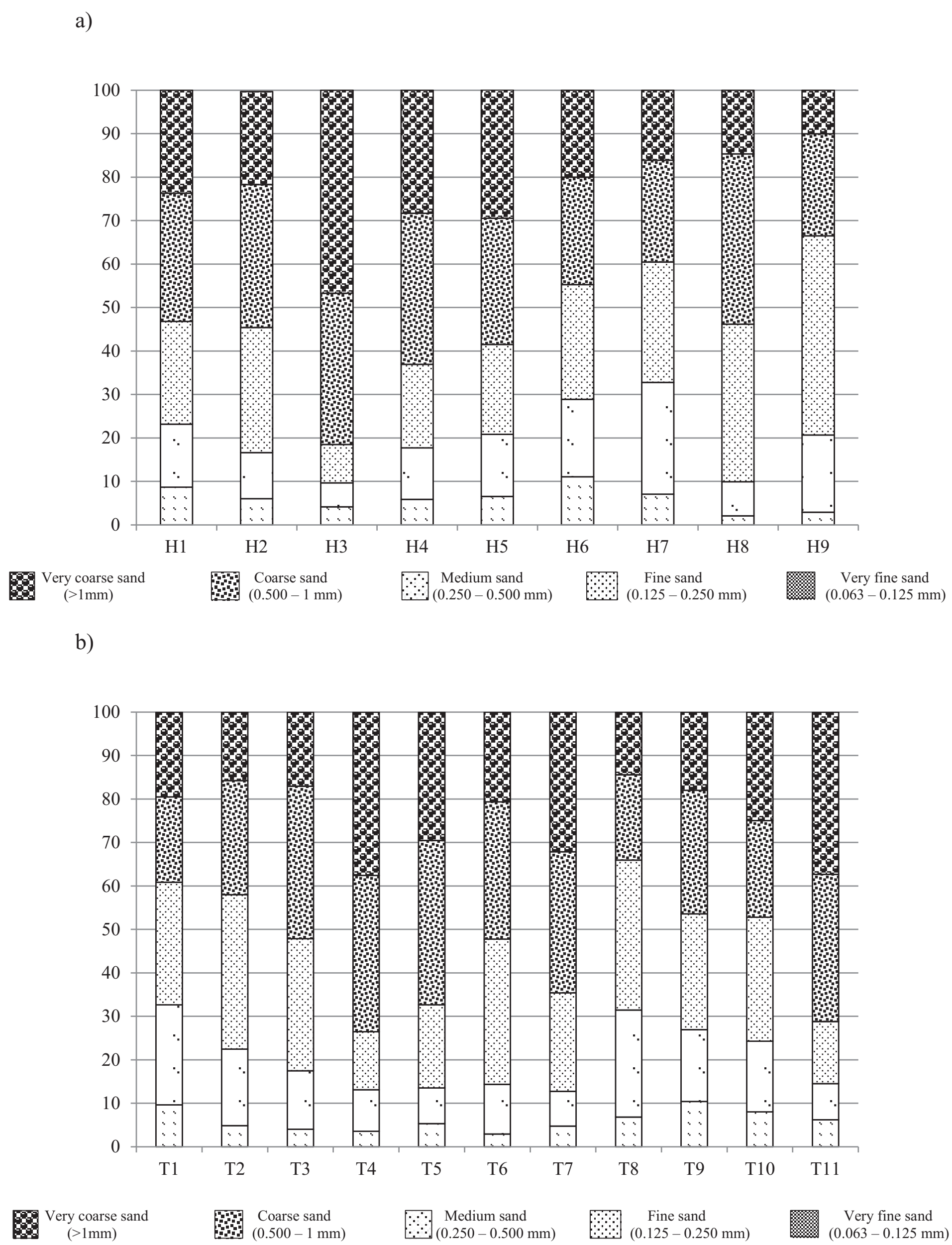

Figure 2. Spatial distribution for the averaged values (February-May 2012) of the granulometry fractions (in %) along the hyporheic flow path of the Henares and the Tajuña Rivers. a) Henares River; b) Tajuña River. Key: H(1-9) = Henares River sampling points; T(1-11) = Tajuña River sampling points. *Distribución espacial de los valores medios (Febrero-Mayo de 2012) de las fracciones granulométricas (en %) a lo largo de la zona hiporreica de los ríos Henares y Tajuña. a) Río Henares; b) Río Tajuña. Clave: H(1-9) = puntos de muestreo en el Río Henares; T(1-11) = puntos de muestreo en el Río Tajuña.*

(0.5 mm < Ø < 1 mm); class 3, medium sand (0.25 mm < Ø < 0.5 mm); class 4, fine sand (0.125 mm < Ø < 0.25 mm); and class 5, very fine sand (0.063 mm < Ø < 0.125 mm). To calculate the percentage of sediments fractions at each site, the weights of the analysed sediments were standardized to 26 g.

The sediment fractions between 0.5 and 0.063 mm were further retained to measure the organic matter content by the loss on ignition (LOI) method (Heiri *et al.*, 2001). The sediments were burned at 550 °C for 24 h in a muffle furnace (Heron, 8E-11). When calculating the percentage, the weight of the sediments before burning was standardised to 8 g. Two replicates of each sample were used.

Statistical analysis

The environmental data were square root-transformed prior to the statistical analyses to meet the assumption of normality (tested by the Shapiro-Wilks test of normality).

The species richness was calculated for each site using the DIVERSE subroutine in the Primer (v.6) statistical software (Clarke & Warwick, 2001). Multivariate distance-based redundancy analyses (db-RDA) were used to determine the ordination of the sampling sites based on the sediment configurations (granulometry), organic matter content (as LOI) and water environmental parameters (NPOC, TOC, BOD_5 and COD). The environmental data were superimposed onto the db-RDA ordination as vectors to examine the contribution of the individual parameters to the dissimilarity of the clusters. The relationships between the 10 environmental variables and the abundance of hyporheic species of cyclopoids and ostracods were explored through canonical correlation analyses (CCA, Hammer *et al.*, 2001) using the PAST 2.6 software. Additionally, Spearman rank correlations were applied to test the associations between the ranked environmental and biotic variables using the STATGRAPHICS® Centurion XV software (StatPoint Technologies, Inc., 2006). The significance of the differences in the parameters describing the cyclopoid and ostracod assemblages between both rivers and the two investigated seasons was determined by applying a Mann-Whitney U test in STATGRAPHICS® Centurion XV.

RESULTS

Environmental parameters in the hyporheic zone

An overview of the mean values of the environmental parameters measured at each sampling site is provided in Supplementary information Table S1. The Mann-Whitney U test ($p < 0.05$) results indicated that the physicochemical parameters of the surface waters (Table 2a) and hyporheic waters (Table 2b) of each stream varied significantly between the two temporal replicates and at the spatial scale. In Henares, the differences in the temperature, EC, pH, NPOC, TOC, COD and SO_4^{2-} (Mann-Whitney U test, $p < 0.05$, Table 2b) of the hyporheic waters among the sampling campaigns were statistically significant. In the Tajuña hyporheic waters, only the temperature, DO, CO_3^{2-} and TOC presented significant differences (Table 2b). In the riverbed sediments, a greater accumulation of organic matter as LOI was detected in the winter season in both streams, although this difference was not statistically significant (Mann-Whitney U test, $p > 0.05$).

Between the hyporheic waters of the two study streams, the EC, temperature and SO_4^{2-} presented the most marked physicochemical variations (see Supplementary information Table S1). The values of temperature, EC and COD were higher in Henares, whereas the values obtained for SO_4^{2-} and DO were higher in Tajuña. The Mann-Whitney U test ($p < 0.05$) results also indicated that the concentrations of NPOC, TOC, COD, EC and HCO_3^- in the interstitial waters of Henares were significantly higher (Table 2b).

The organic matter content in the sediments slightly varied between both streams (overall mean values of 5.47% in Henares and 4.88% in Tajuña), but the difference was not statistically significant ($p > 0.05$).

Table 2. Mann-Whitney U test results for the comparison of the physicochemical studied parameters between and within the studied rivers. a) Comparison of surface waters; b) comparison of hyporheic waters. Key: * = parameter that presents significant differences ($p < 0.05$). Abbreviations: T = temperature; EC = electrical conductivity; DO = dissolved oxygen; BOD_5 = biological oxygen demand; COD = chemical oxygen demand; NPOC = non-purgeable organic carbon; TOC = total (dissolved) organic carbon; TC = total carbon; IC = inorganic carbon. *Resultado del test U de Mann-Whitney realizado para la comparación entre ríos y en cada uno de ellos de los parámetros fisicoquímicos estudiados. a) comparación en aguas superficiales; b) comparación en aguas hiporreicas. Clave: * = parámetro estadísticamente diferente* (p < *0.05). Abreviaturas: T = temperatura; EC = conductividad eléctrica; DO = oxígeno disuelto;* BOD_5 *= demanda biológica de oxígeno; COD = demanda química de oxígeno; NPOC = carbono orgánico no purgable; TOC = carbono orgánico total; TC = carbono total; IC = carbono inorgánico.*

a)

U-test values for the comparison of the studied physicochemical parameters (surface waters)

	Henares	Tajuña	Henares-Tajuña	Henares-Tajuña	Henares-Tajuña
	Between surveys	Between surveys	Survey 1	Survey 2	Both surveys
U-test	U-test	U-test		U-test	U-test
T (°C)	40.5*	−60.5*	−31.5*	−14.5	−46.0
EC	−31.5*	3.5	−33.5*	−23.5	−104.0*
pH	−3.0	30.5*	22.5	9.5	67.0
DO	−23.0*	37.5*	20.0	25.0	81.0*
BOD_5	0.0	−27.0	−4.5	22.5	36.0
COD	34.5*	−60.5*	−21.5	−5.5	−27.0
NPOC	15.5	−18.5	−31.0*	−39.5*	−137.5*
TOC	32.5*	−56.0*	−45.5	−40.0*	−138.0*
TC	−20.5	41.5*	22.0	21.0	−74.0*
IC	−24.5*	43.5*	28.5*	23.5	88.0*
CO_3^{2-}	1	2.5	3.5	1.5	9.5
HCO_3^-	−13.5	28.5	18.5	17.5	73.0*
SO_4^{2-}	−30.5	−10.5	−22.5	−14.5	−72.0

b)

U-test values for the comparison of the studied physicochemical parameters (hyporheic waters)

	Henares	Tajuña	Henares-Tajuña	Henares-Tajuña	Henares-Tajuña
	Between surveys	Between surveys	Survey 1	Survey 2	Both surveys (average)
	U-test	U-test	U-test	U-test	U-test
T (°C)	40.5*	−60.5*	−30.5*	−18.0	−48.5
EC	−31.5*	1.5	−25.5	−17.5	−75.0*
pH	−10.0*	23.5	6.0	3.5	18.5
DO	−22.5	33.0*	7.5	21.0	56.5
BOD_5	20.5	−7.5	29.0*	1.5	58.0
COD	39.5*	10.5	−45.5*	−48.5*	−168.0*
NPOC	24.0*	−24.5	−49.5*	−35.5*	−150.5*
TOC	29.5*	−44.0*	−49.5*	−36.5*	−150.5*
TC	−10.5	13.0	20.0	16.0	72.5
IC	−13.5	16.5	30.5*	19.5	93.0*
CO_3^{2-}	−3.5	28.5*	18.5	−1.5	31.0
HCO_3^-	−11.5	−0.5	19.5	19.5	83.0*
SO_4^{2-}	−31.5*	−1.5	−13.5	−13.5	−55.0
LOI	−7.0	− 3.5	−14.5	−6.5	− 43.0

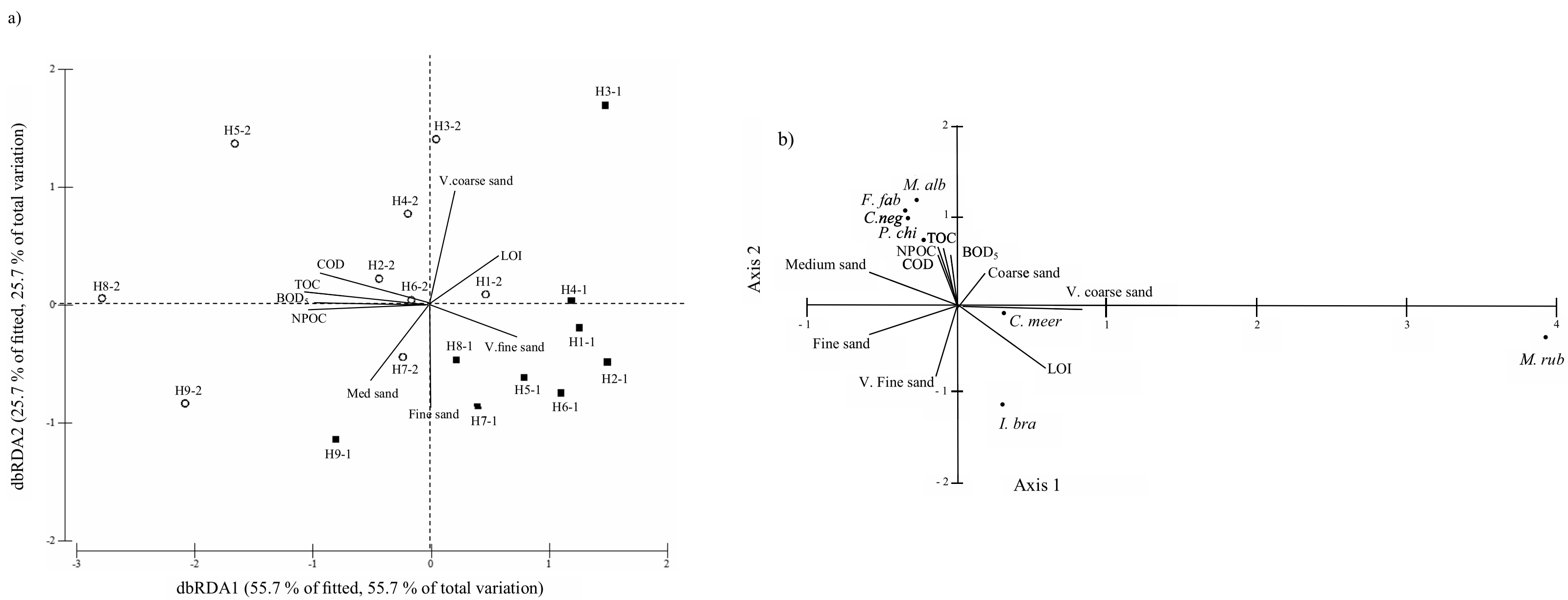

Figure 3. Multivariate analyses results for the Henares River. a) Results for the db-RDA (Euclidean distance) and vectors (Spearman rank correlation $R > 0.5$) considering 10 environmental parameters [BOD_5, COD, TOC, NPOC, very coarse sand fraction, coarse sand fraction, medium sand fraction, fine sand fraction, very fine sand fraction, and organic matter content in sediments (LOI)]. b) Results for the CCA showing the distribution of the most relevant species and their relationship with the 10 studied environmental parameters [BOD_5, COD, TOC, NPOC, very coarse sand fraction, coarse sand fraction, medium sand fraction, fine sand fraction, very fine sand fraction, and organic matter content in sediments (LOI)]. Key: black squares = wintertime samples (February 2012); hollow circles = springtime samples (May 2012); black points = species. Abbreviations: COD = chemical oxygen demand; NPOC = non-purgeable organic carbon; BOD_5 = biological oxygen demand; TOC = total (dissolved) organic carbon; LOI = loss on ignition-based organic matter content in sediments; V. fine sand = very fine sand fraction; Med. sand = medium sand fraction; V. coarse sand = very coarse sand fraction; H(1-9) = Henares River sampling points; *P. chi* = *Paracyclops chiltoni*; *C. neg* = *Candona neglecta*; *M. alb* = *Macrocyclops albidus*; *F. fab* = *Fabaeformiscandona fabaeformis*; *I. bra* = *Ilyocypris brady*; *C. meer* = *Candona meerfeldiana*; *M. rub* = *Microcyclops rubellus*. *Resultados de los análisis multivariados en el Río Henares. a) Resultados del db-RDA (distancia Euclidea) y vectores (correlacion de rango de Spearman,* R *> 0.5) considerando 10 parámetros ambientales (BOD_5, COD, TOC, NPOC, fracción de arenas muy gruesas, fracción de arenas gruesas, fracción de arenas medias, fracción de arenas finas, fracción de arenas muy finas, y contenido de materia orgánica en sedimentos, LOI). b) Resultados del CCA mostrando las especies más relevantes y su relación con los 10 factores ambientales estudiados (BOD_5, COD, TOC, NPOC, fracción de arenas muy gruesas, fracción de arenas gruesas, fracción de arenas medias, fracción de arenas finas, fracción de arenas muy finas, y contenido de materia orgánica en sedimentos, LOI). Clave: cuadrado negro = muestras de invierno (Febrero 2012); circulo vacío = muestras de primavera (Mayo 2012); puntos negros = especies. Abreviaturas: COD = demanda química de oxígeno; NPOC = carbono orgánico no purgable; BOD_5 = demanda biológica de oxígeno; TOC = carbono orgánico total; LOI = contenido de materia orgánica en sedimento medido mediante el método de loss on ignition; V. fine sand = fracción de arenas muy finas; Med. sand = fracción de arenas medias; V. coarse sand = fracción de arenas muy gruesas; H(1-9) = puntos de muestreo en el Río Henares;* P. chi = Paracyclops chiltoni*;* C. neg = Candona neglecta*;* M. alb = Macrocyclops albidus*;* F. fab = Fabaeformiscandona fabaeformis*;* I. bra = Ilyocypris brady*;* C. meer = Candona meerfeldiana*;* M. rub = Microcyclops rubellus.

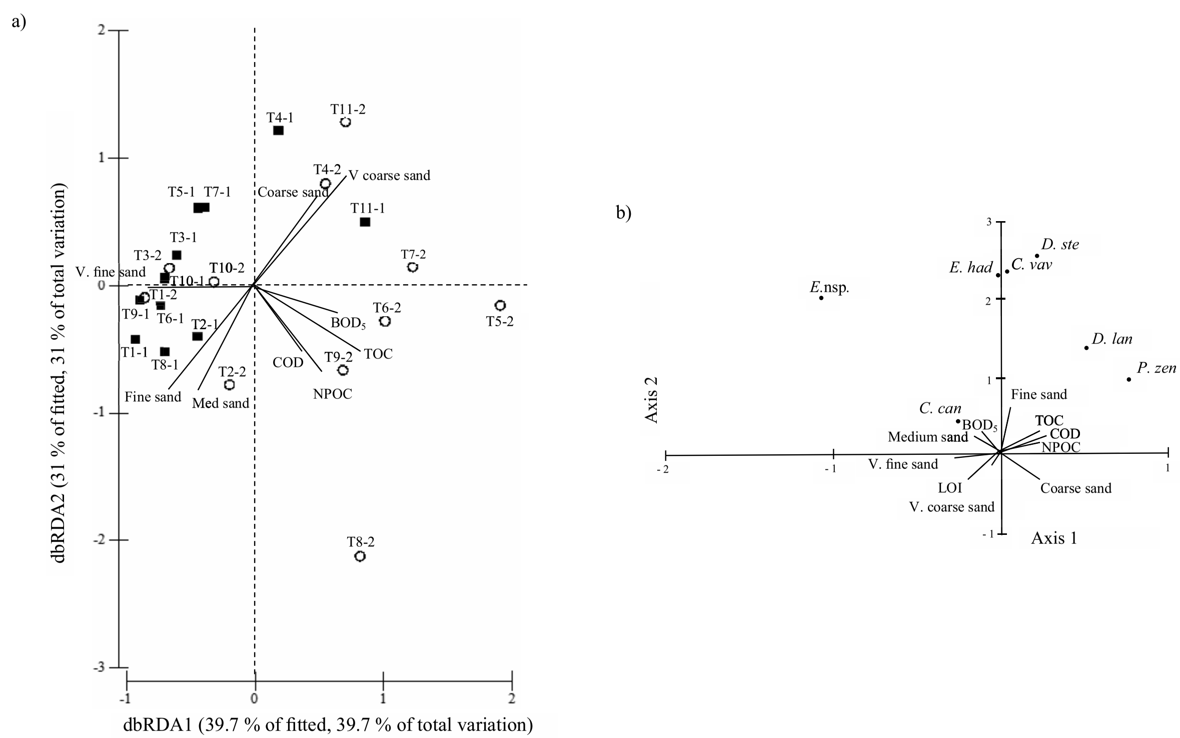

Figure 4. Multivariate analyses results for the Tajuña River. a) Results for the db-RDA (Euclidean distance) and vectors (Spearman rank correlation $R > 0.5$) considering 10 environmental parameters [BOD_5, COD, TOC, NPOC, very coarse sand fraction, coarse sand fraction, medium sand fraction, fine sand fraction, very fine sand fraction, and organic matter content in sediments (LOI)]. b) Results for the CCA showing the distribution of the most relevant species and their relationship with the 10 studied environmental parameters [BOD_5, COD, TOC, NPOC, very coarse sand fraction, coarse sand fraction, medium sand fraction, fine sand fraction, very fine sand fraction, and organic matter content in sediments (LOI)]. Key: black squares = wintertime samples (February 2012); hollow circles = springtime samples (May 2012); black points = species. Abbreviations: COD = chemical oxygen demand; NPOC = non-purgeable organic carbon; BOD_5 = biological oxygen demand; TOC = total (dissolved) organic carbon; LOI = loss on ignition-based organic matter content in sediments; V. fine sand = very fine sand fraction; Med. sand = medium sand fraction; V. coarse sand = very coarse sand fraction; T(1-11) = Tajuña River sampling points; *P. zen* = *Pryonocypris zenkeri*; *D. lan* = *Diacyclops languidoides languidoides*; *E. had* = *Eucyclops hadjebensis*; *D. ste* = *Darwinula stevensoni*; *C. can* = *Candona candida*; *E. nsp* = *Eucyclops* n. sp.; *C. vav* = *Cryptocandona vavrai*. *Resultados de los análisis multivariados en el Río Henares. a) Resultados del db-RDA (distancia Euclidea) y vectores (correlacion de rango de Spearman,* R > *0.5) considerando 10 parámetros ambientales (BOD_5, COD, TOC, NPOC, fracción de arenas muy gruesas, fracción de arenas gruesas, fracción de arenas medias, fracción de arenas finas, fracción de arenas muy finas, y contenido de materia orgánica en sedimentos, LOI). b) Resultados del CCA mostrando las especies más relevantes y su relación con los 10 factores ambientales estudiados (BOD_5, COD, TOC, NPOC, fracción de arenas muy gruesas, fracción de arenas gruesas, fracción de arenas medias, fracción de arenas finas, fracción de arenas muy finas, y contenido de materia orgánica en sedimentos, LOI). Clave: cuadrado negro = muestras de invierno (Febrero 2012); circulo vacío = muestras de primavera (Mayo 2012); puntos negros = especies. Abreviaturas: T = temperatura; COD = demanda química de oxígeno; NPOC = carbono orgánico no purgable; BOD_5 = demanda biológica de oxígeno; TOC = carbono orgánico total; LOI = contenido de materia orgánica en sedimento medido mediante el método de loss on ignition; V. fine sand = fracción de arenas muy finas; Med. sand = fracción de arenas medias; V. coarse sand = fracción de arenas muy gruesas; T(1-11) = puntos de muestreo en el Río Tajuña;* P. zen = Pryonocypris zenkeri*;* D. lan = Diacyclops languidoides languidoides*;* E. had = Eucyclops hadjebensis*;* D. ste = Darwinula stevensoni*;* C. can = Candona candida*;* E. nsp = Eucyclops *n. sp.;* C. vav = Cryptocandona vavrai.

For both rivers, a significant longitudinal pattern was detected along their hyporheic flow paths (spatial scale) for the selected environmental parameters. The high mineralization and EC in the lower sectors of both rivers were quite evident (H6-H9 and T8-T11) due to the high contents of Ca^{2+} and Mg^{2+}, which are characteristic of hard waters (see Supplementary information Table S1). The lower sector of Tajuña (T8-T11) was characterized by calcium sulphate-rich waters, which presented threefold-higher levels than those observed upstream due to the presence of gypsum-sulphate rocks (see also Iepure *et al*., 2014). An increment in NPOC was noted in the hyporheic waters of both rivers (Spearman correlation, $R = -0.62$, $p < 0.05$) with decreasing elevation. Increases were also observed in the TOC in Henares ($R = -0.51$, $p < 0.05$) and the organic matter content in the sediments in Tajuña ($R = -0.65$, $p < 0.05$) with decreasing elevation. Oppositely, the organic matter content in the hyporheic sediment in Henares was lower when elevation decreased ($R = 0.57$, $p < 0.05$).

Grain size of HZ sediments

Table 1 presents a general overview of the riverbed substrate characteristics at the 20 investigated hyporheic sites, and Figure 2 illustrates the spatial distributions of the grain sizes. The riverbed substrate structure of both streams was virtually similar in terms of grain size classes (Mann-Whitney U test, $p > 0.05$). The percentage of very fine fractions (0.125<Ø< 0.063 mm) in both rivers was higher in winter, whereas the percentages of the very coarse (Ø > 1 mm) and coarse sand fractions (0.5 mm < Ø < 1 mm) were higher in spring.

An alteration in the sediment grain size distribution along the longitudinal transect was detected in both streams (Fig. 2). In Henares, the percentage of the very coarse fraction positively correlated to the elevation ($R = 0.63$, $p < 0.05$), whereas the medium sand fraction showed the opposite relationship ($R = -0.54$, $p < 0.05$) (Fig. 2a). Conversely, in the Tajuña River, several successive alterations in the substrate composition in terms of grain size distribution were detected; i.e., at upstream T5, the riverbed substrates were dominated by the very coarse (Ø> 1 mm) and coarse fractions (1< Ø > 0.5 mm), whereas the percentages of the finer fractions (Ø < 0.5 mm) increased downstream at sites T5 and T10 (Fig. 2b).

Abundances and diversity of Ostracoda and Cyclopoida

In all, 2466 ostracod and cyclopoid individuals were collected from the HZ of both streams (1872 individuals in Tajuña and 594 specimens in Henares) (see Supplementary information Table S2, available at www.limnetica.com). Thirty-one species and subspecies (25 species in Tajuña and 23 in Henares) were identified. From them, 28 were ecologically classified as non-stygobites and three as stygobites (*Eucyclops hadjebensis, Acanthocyclops venustus* gr., and *Acanthocyclops* n. sp.).

The number of species and the population density of hyporheic cyclopoids and ostracods did not vary significantly over time between the two rivers (Mann-Whitney U test, $p > 0.05$). However, both groups showed discontinuities along the hyporheic flow path in terms of the density and diversity of the populations and species turnover; e.g., along the Henares River, the maximum density was reached in the upstream sector at H2 (110 individuals, 5 species), with *Diacyclops languidoides languidoides* and *Darwinula stevensoni* contributing significantly to the increase in the density of the hyporheic population (see Supplementary information Table S2). Along the Tajuña River, the densities and species diversity of cyclopoids and ostracods also showed a strikingly discontinuous pattern along the hyporheic flow path, with a distinctly marked difference between the sites upstream of T8 (1460 individuals, 19 species) and the sites downstream of this site (412 individuals and 13 species) (see Supplementary information Table S2). The maximum densities in Tajuña were detected at several sites: at T2, where the maximum densities of *Paracyclops imminutus* and *Potamocypris fulva* were detected; at T4, where high densities of *Paracyclops fimbriatus* and *Potamocypris fulva* were observed; and at T10 where, the maximum densities of *Acanthocyclops venustus* gr. were identified (see Supplementary information Table S2).

Relationship between the environmental conditions and the meiofauna

The first two axes of db-RDA for the Henares stream explained 81.4% of the fitted variation, and the first axis showed major significance and separated the sites at a temporal scale (Fig. 3a). In winter, higher concentrations of EC, SO_4^{2-}, NPOC, TC, IC and CO_3^{2-} and high contents of the fine sand fraction and organic matter content characterised the water conditions and substrates of the Henares HZ. The CCA accounted for 48% of the total variance of the species data and highlighted the relationship between the environmental factors and distinct hyporheic species assemblages in the Henares River. The first axis of the CCA results were primarily an increasing gradient of the very coarse sand (> 1 mm), the coarse sand fractions (0.5-1 mm), and the organic content (LOI) accounting for 18.73% of the species-environment relationships. *Acanthocyclops robustus* and *Eucyclops hadjebensis* had low scores on this first axis, indicating that they tended to be found in areas with low amounts of very coarse sands, whereas *Microcyclops rubellus* presented high scores on this axis being mainly related to the organic matter content (LOI) (Fig. 3b). The second axis was primarily an increasing gradient of the coarse sand fraction (0.5-1 mm) and accounts for an additional 18.54% of the species-environment relationships (Fig. 3b). The ostracod *Pryonocypris zenkeri* had a high score on this axis, suggesting that it is mainly found in coarse hyporheic sediments. Spearman rank correlation analyses supported some of the trends indicated by the CCA results, i.e., *Microcyclops rubellus* correlated significantly with a high organic matter content ($R = 0.6, p < 0.05$) and a high percentage of very coarse sands ($R = 0.85$, $p < 0.05$), *Paracyclops chiltoni* was associated with a high proportion of the medium sand fraction ($R = 0.55$, $p < 0.05$), and *Macrocyclops albidus* was also related to a high proportion of the medium sand fractions ($R = 0.51, p < 0.05$).

For Tajuña, the first two axes of the db-RDA explained 70.7% of the total variance (Fig. 4a) and similarly distinguished the sites according to the temporal variations in the environmental conditions. However, the level of distinctness was lower compared with that found for Henares. The CCA results (explaining 46.16% of the total variance) indicated that an increase in the proportion of coarse sands (0.5-1 mm) was markedly favourable for *Paracyclops chiltoni* and that a decrease in the proportion of the medium sand fractions (0.25-0.5 mm) was favourable for *Candona candida* and *Pseudocandona albicans* (Fig. 4b). Conversely, the second axis was mainly an increasing gradient of the fine sand fraction (0.25-0.125 mm) and accounted for 20.07% of the variance in the species-environment relationships (Fig. 4b). *Eucyclops hadjebensis*, *Cryptocandona vavrai* and *Darwinula stevensoni* presented high scores on this axis, suggesting that these taxa were mainly found in substrates with a high proportion of fine sediments. Spearman correlations indicated significant correlations only for *Cryptocandona vavrai*, which was positively related to the fraction of fine sediments ($R = 0.44$, $p < 0.05$), and *Pryonocypris zenkeri*, which was negatively correlated with the organic matter content ($R = -0.5$, $p < 0.05$).

DISCUSSION

Effects of granulometry and organic matter content on ostracod and cyclopoid distribution

The data presented herein represent the first published attempt to compare the diversity (richness) and distribution of cyclopoids and ostracods in the HZ of two Mediterranean groundwater-fed streams of the Jarama basin with contrasting geological compositions: carbonates (Tajuña River) *vs.* siliceous (Henares River). This study was based on the assumption that the riverbed lithology and sediment granulometry create heterogeneity in the physicochemical features along the longitudinal hyporheic flow path (Brunke & Gonser, 1997; Harvey *et al.*, 2012), which would alter the diversity and distribution of the hyporheic biota (Dole-Oliver *et al.*, 2009, Gibert *et al.*, 1994; Martin *et al.*, 2009; Marmonier *et al.*, 2012). Alterations in the physicochemistry of both rivers have been previously reported (Iepure *et al.*, 2013) and provide complementary information to depict the water conditions and origin in the HZ. Although the riverbeds of both rivers contained different geological materials, the Mann-Whitney U test did not indicate any significant differences in the structural texture between the siliceous substrate of the Henares and the carbonate substrates of the Tajuña. However, the multivariate db-RDA analysis indicated a clear distinction in the distribution of the grain size classes along the hyporheic flow path.

In Henares, the distribution of the major grain size sediments classes was more homogenous, as characterized by the slight predominance of medium and fine sands over coarse sands throughout the entire riverbed, which led to a smaller pore volume and a reduced permeability of hyporheic zone substrates (Arribas *et al.*, 2000). These facts would hinder surface exchange processes, the slightly higher residence time of water in riverbed sediments and, subsequently, poorer hyporheic water renewal. Therefore, although the concentration of organic matter in the sediments of Henares was higher than that observed in Tajuña, the low riverbed permeability of Henares hampered the development of large cyclopoid and ostracod populations (see also Gibert & Deharveng, 2002; Descloux *et al.*, 2013). Nevertheless, in Henares, the main deterministic variables responsible for the presence of cyclopoids and ostracods were the low amounts of medium and coarse sands (i.e., favourable for *Acanthocyclops robustus* and *Eucyclops hadjebensis*) (Fig. 3b).

Alongside Tajuña, the riverbed substrates were dominated by the very coarse and coarse sand fractions (Ø > 0.5 mm) as opposed to fine sands (0.125 < Ø < 0.250 mm). The texture of these types of sediments, which comprise large pore spaces, is associated with a high permeability and consequently offers suitable shelter habitats for hyporheic crustaceans. These features further amend not only the residence time of water in the HZ but also the dynamics of organic matter, such as its retention and input into sediments (Franken *et al.*, 2001; Worral & Burt, 2004; Ran *et al.*, 2013). The HZ of Tajuña River was found to be present a significantly more diverse and abundant population of crustaceans, particularly cyclopoids, compared with that observed in Henares. The sediment fraction formed by coarse sands in the hyporheic zone of Tajuña River aided the maintenance of large species such as *Paracyclops chiltoni,* whereas fine sands (0.125-0.25 mm) were found to be mainly favourable for *Eucyclops hadjebensis*, *Cryptocandona vavrai* and *Darwinula stevensoni* (Fig. 4b). Nevertheless, the relationships between the distinct grain size classes and the organic matter content in the sediments were weak. Previous research conducted in Tajuña has certainly indicated that the higher cyclopoid and ostracod densities in the upper sectors of the river were more related to the water physicochemistry, mainly due to the presence of carbonates; whereas the presence of gypsum-sulphates in the lower sector partially explained the observed scarcity or lack of species (Iepure *et al.*, 2013, 2014). Our data also revealed that cyclopoids and ostracods were not uniformly distributed along the hyporheic flow path of Tajuña, which presented sectors of increasing population densities and diversities followed by abrupt decreases. This pattern indicates heterogeneity along the longitudinal transect, expressed by short-distance alterations to the hyporheic riverbed structure, as indicated by the grain size distribution (Fig. 2b). These discontinuities in the structural pattern of the hyporheic riverbed may be associated with the hyporheic habitats disruption, likely related to the presence of artificial dams along the river course, which are assumed to induce fluctuations in the river flow, geohydrochemical dynamics and thermal changes downstream (Iepure *et al.*, 2014).

The within-rivers differences, as indicated the db-RDA, were found to be shaped by temporal changes in the sediment texture and organic content rather than spatial changes. A considerably more significant temporal distinction was found in the Henares River compared with Tajuña, resulting in a greater discrepancy among the sites of the two surveys. In winter, the hyporheic riverbed sites of Henares (except site H3) were characterised by a predominance of fine sediments (i.e., very fine and fine sands) and by a relatively high organic matter content, which accumulated in the stream bed sediments due to the base flow of the river. These tended to arrest the development of communities, particularly in terms of diversity, which was slightly higher in winter than in spring (19 *vs.* 15 species). Similarly, the density remained low in both seasons. The hyporheic communities of the Henares River were mirrored in winter by the dominance of species with large ecological valences and strong thermal tolerances, such as *Microcyclops rubellus* and *Diacyclops languidoides languidoides* among cyclopoids and *Candona neglecta* among ostracods (Reid *et al.*, 1991; Pesce, 1994; Särkkä *et al.*, 1998; Mezquita *et al.*, 1999). In Henares, a marked shift toward a predominance of the medium to very coarse sand fractions was observed in springtime and was found to be associated with increased water channel discharge, which could restructure the sediments, deplete the sediment detritus and slightly increase the NPOC and DOC concentrations on the hyporheic waters (Worral & Burt, 2004; Ran *et al.*, 2013). This latter augmentation could also be associated with the high river productivity observed during this period. Consequently, the community structures of cyclopoids and ostracods in the Henares River changed slightly, and the population densities, particularly that of cyclopoids, increased. Among these, large species with sizes up to 1 mm (cf. Maier, 1994; Meisch, 2000; Dussart & Defaye, 2001), such as *Macrocyclops albidus* and *Paracyclops chiltoni*, were well-represented, whereas the most dominant ostracods were *Herpetocypris brevicaudata* and *Fabaeformiscandona fabaeformis*.

In the Tajuña River, seasonal changes were also evident, but not as pronounced as those detected in Henares. The fine sediments fractions were more abundant in the low flow of winter, whereas a shift toward coarser and very coarse sands occurred in spring, when the discharge to Tajuña increased almost twofold. Consequently, the partition of the hyporheic community structure followed similar trends to those observed in Henares in terms of diversity, which was higher in winter, and density, which increased in spring. In Tajuña, several large populations of stygobites were detected and were represented by three species that are endemic to the Iberian Peninsula: i.e., *Acanthocyclops venustus* gr. and *Acanthocyclops* n. sp.; and to the Balearic Islands: i.e., *Eucyclops hadjebensis* (Gourbault & Lescher-Moutoué, 1979); and frequently observed stygophile hyporheos ostracod *Cryptocandona vavrai* (see Supplementary information Table S2). Our findings confirm the previous assumption that a more significant upwelling would occur along the entire course of the Tajuña River, which would contribute to the shaping of hyporheic communities and to the sustaining of stable populations of obligate groundwater dwellers (Iepure *et al.*, 2013). The patchy habitat heterogeneity in the Tajuña imprinted by substrate discontinuities contributed substantially to the sparse species distribution along the entire river course resulting in evident species turnover among sites, as reflected by the presence of single-site species, such as *Candona vavrai* and *Eucyclops hadjebensis* (at T1), *Acanthocyclops robustus* (at T9) and *Paracyclops chiltoni* (at T10). Noticeable dam-induced changes in the HZ communities of the Tajuña River were evident for a few species, as reflected by the smaller *Paracyclops fimbriatus* populations located downstream of the artificial reservoir (at T4). However, the opposite effect was obtained for *Acanthocyclops venustus* gr. (see Supplementary information Table S2).

CONCLUSIONS

The lithology and organic matter content in alluvium sediments of the HZ and the presence of cyclopoid and ostracod crustaceans were used to describe the hyporheic zone conditions of two Mediterranean rivers in central Spain. At the temporal and spatial scales, the composition of hyporheic cyclopoids and ostracods reflected the distribution and dominance of the grain size classes of the sediments and helped identify zones with distinct diversity and abundance. Although the observed diversity and abundance were altered mainly by the heterogeneity of the riverbed substratum along the hyporheic flow path and by the water physicochemistry, the organic matter loads of the sediments did not play a significant role. Changes in the cyclopoid and ostracod communities also indicated distinctness in the surface-subsurface water interactions since diversity of species with distinct ecological valences (stygobites *vs.* non-stygobites) increased in a more active HZ river channel, triggered by a riverbed dominated by coarse sediments of carbonate rocks that exhibited a higher potential of presenting upwelling spots along its flow path. The differences in the hyporheic assemblage composition and diversity between both rivers with contrasting geology indicated that in the same hydrographic basin, the hyporheic biota is a useful indicator of changes in the physical riverbed substrate structure. Further efforts to understand the factors responsible for the hyporheic fauna diversity and distribution in distinct typological categories of rivers will improve our understanding of the responses of the HZ to natural and anthropogenic disturbances.

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