ANTHROPIC EFFECTS ON THE MEIOFAUNA AND PHYSICOCHEMICAL CHARACTERISTICS OF THE HYPORHEIC ZONE IN A MEDITERRANEAN STREAM

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ABSTRACT

We examined the effects of the discharge from a wastewater treatment plant (WWTP) on the interstitial water chemistry and structure of the hyporheos in two sections of the La Tordera stream, one upstream and one downstream of the effluent from the treatment plant. Interstitial water samples were collected to determine the vertical hydraulic gradient (VHG) and hydraulic conductivity (K) at six sites in each of the two sections on six occasions between November 2006 and September 2007. Samples of the interstitial meiofauna were collected in upwelling zones in both sections of the river. There were no patterns in the hydraulic gradient either in its direction or its magnitude along the longitudinal axis. There were no differences in VHG recorded at the sites sampled indicating that the two sections were similar in terms of geomorphology. The hydraulic conductivity was greatest in the control section (upstream of the effluent) of the stream. There were no significant differences in specific conductance, phosphates, nitrates or ammonia in the upwelling and downwelling zones. In the upwelling zones in the section of the river downstream from the effluent the concentrations of nitrates were greater than in the downwelling zones, but levels of phosphates and ammonia were lower. There was a decrease in the interstitial nutrient concentrations along a longitudinal gradient downstream of the effluent. A total of 24 taxa were collected from the hyporheos, with Chironomids and Oligochaetes being the most abundant groups. A higher Shannon diversity index (2.86) was recorded in the downstream section of the river even though a greater taxa richness (18) was recorded in the control section. This is probably due to the fact that a larger number of rare or unusual species was found in this section. According to the BMWP biological index the water quality in the control and downstream sections was polluted and heavily polluted, respectively. The shifts in hydraulic exchange processes and the bio-physicochemical environment of the hyporheic zone are the main effects of the discharge from the Santa Maria Palautordera wastewater treatment plant on the two sections of the river studied.

Keywords: point source, invertebrates, hyporheic zone, hydraulic gradient, BMWP index

Introduction

Fluvial ecosystems in developed regions are subject to high environmental stress due to eutrophication (Fisher et al. 1998). Human activities may cause land use changes in catchment areas or modifications of the landscape that increase the transport of nutrients towards aquifers and surface water. Water courses (rivers and streams) may also be directly affected by discharges of waste water (point sources) and other modifications, which reduce their capacity to respond to an increase in nutrient concentrations. These problems are accentuated in Mediterranean rivers due to the persistent scarcity of water (Martí and Sabater 1996; Cushing and Allan 2001). Point sources of pollution of surface water bodies, for example, discharges from water treatment plants (sewage), industrial installations or high intensity precipitations, which frequently contribute high loads of pollution that affect the quality of river water, are an expected or derived consequence of urban and industrial development. In addition, and depending on the relative volumes of the effluent and the river, the discharge from a sewage works can represent an important percentage of the river volume downstream of the effluent. These factors act at different scales ranging from a particular stretch of a river to the whole catchment area. Contaminants in fluvial ecosystems may affect the quality of the groundwater in several ways: firstly, in those areas where the river loses water to the aquifer (downwelling); secondly, along sections where the aquifer is very deep, favouring the infiltration of surface water, and thirdly, during periods of flooding when the river water spreads over the floodplain. The concentrations of these contaminants may, however, be reduced by biogeochemical processes, which take place in shallow aquifers and the hyporheic zone (Boulton et al. 1997; Fuller and Harvey 1999; USGS 2001).

Since 1950 aquatic ecologists have emphasized the importance of the interaction between surface water and groundwater in the functioning of aquatic ecosystems. The region where the water in the aquifer and the river mix is known as the hyporheos and is important in that in its sediments there are diverse, metabolically active microbial communities, which retain and transform essential biological elements. There are few studies on the influence of the hyporheos on fluvial ecosystem metabolism, but some conceptual models propose that this depends on the type and metabolic rate of the microbial communities, the proportion of the river volume that flows through them and their influence on hydrological residence times (Triska et al. 1989a; Fellows et al. 2001). The hyporheic zone can have a very dynamic flow regimen. At maximum volume or *flooding peaks*, water flows from the surface to the subsurface, recharging the aquifer. At low volumes, however, water flows from

the aquifer into the river, providing the main contribution to the basal volume of most rivers. The recharge and discharge zones are more abundant where the bottoms of rivers consist of coarse sediments rather than sand. Studies of the changes in fluvial geomorphology along a longitudinal profile have produced a model in which areas of recharge (*downwelling*) alternate with areas of discharge (*upwelling*). When the depth of the water channel decreases, high pressure zones are produced where the river bed starts to rise (*riffle*), into which water flows, displacing the interstitial water and flowing under the river bed to the opposite end of the riffle where the increase in depth produces a low pressure zone (*pool*) and the water in the hyporheos comes to the surface (*upwelling* zone). The alternating flow model describes a gradient of physicochemical conditions between the river and groundwater, where biochemical processes, together with the hydraulic residence time in the zone, also interact. In general, the concentration of nutrients in the hyporheic water is significantly higher than in the surface water. Given the importance of nutrients in production processes, a reserve of these in the hyporheos could be of fundamental importance in the metabolic processes of fluvial ecosystems (Sabater and Vila 1991; Franken et al. 2001).

Nutrient retention is a function of the fluvial ecosystems that could contribute to the recovery of water quality in contaminated rivers. This is governed by the interaction between physicochemical and biological factors, among the most important being the topography of the river bed, the thickness of the hyporheic layer, hydrological factors, etc. Depending on prevailing conditions, however, some of these factors will take precedence over others. The exchange of water and nutrients in the hyporheic zone results in a reduction in the transport of certain elements, whether due to physicochemical transformations or microbiological absorption, making this exchange zone, or surface/subsurface transition zone of vital importance for the optimization of the auto-regenerating capacity of those rivers considered to be pristine. Some years ago, Jones and Holmes (1996) reported that the underlying hyporheic zone acted as a source of nitrates in nitrogen deficient streams, but as a sink in streams with high nitrogen concentrations. In addition, Hill et al. (1998) suggest that in streams with high nitrogen concentrations the retention of nitrates is particularly affected by the size of the surface water retention zone. A larger retention zone has a longer residence time, resulting in increased contact with those sites adjacent to the surface/subsurface interphase where the exhaustion of nitrates occurs. This capacity for physical and biological nutrient retention can be modified by changes in the morphology of the river channel or the permeability of the sediment, or by increases in the nutrient load from point sources of contamination, such as effluents from wastewater treatment plants. This increase in the availability of macronutrients, such as nitrogen and phosphorus, produces an increase in the primary productivity of the system and may accelerate the obstruction or clogging of the interstitial spaces in the sediment, consequently reducing the exchange of elements in the surface / aquifer interphase. Taking into consideration these ideas the aim of this study was to evaluate the effects of the effluent from an urban wastewater treatment plant on the meiofauna and physicochemical characteristics of the hyporheic zone along the La Tordera River.

Material and Methods

Study Area

The catchment area of the La Tordera river is located in Cataluña, Northeast Spain, and extends over 868.5 km^2 (Fig. 1). Owing to the Mediterranean climate it has a low average annual precipitation of about 805.6 mm irregularly distributed throughout the year with spring and autumn being the wettest seasons, and an average annual discharge at its mouth of 3.17 m^3 s^{-1}. The hydric balance of the catchment is positive with a net annual gain of between 70 and 80 hm^3 distributed in zones tending towards a deficit and others tending towards an excess. Due to the proximity of the catchment basin to the coast it has been intensively settled and developed, with a population higher than the average for the country. This has resulted in a series of environmental disturbances, such as water pollution, the occupation of the alluvial plain, the destabilization of the natural fluvial dynamics and the overexploitation of surface and underground watercourses (Albiol 2002). The experimental sub-catchment within the La Tordera drainage basin was selected because of the location of a WWTP (Santa María de Palautordera), which discharges polluted water into the river, dividing it into two sub-reaches, upstream (control section) and downstream (altered section) of the effluent, thus permitting us to investigate the effect of the effluent on the functioning of the surface and subsurface ecosystems (Fig. 1). In La Tordera, land use within the sub-catchment located upstream of the section of the river studied (7024 inhabitants and 80.4 km²) there is 87.4% forest and open land, 10.8% arable and grassland and 1.8% urban, of which the latter is concentrated in the lower part of the basin. Each experimental section was approximately 100 m long, with a substrate mainly of cobbles (34%), pebbles (22%) and boulders (22%). The WWTP discharges into La Tordera stream the result of treating 5808 inhabitant-equivalents, where 1 inhabitant-equivalent is the biodegradable organic matter load equivalent to a BOD$_5$ of 60 g O$_2$ day^{-1}. This particular WWTP uses the activated sludge method to treat the waste water but lacks the technology to actively remove nitrogen or phosphorus. As regards the biological communities present, Diatoms dominate the microbiota and the most abundant macroinvertebrate families in the spring months are Baetidae, Ephemerellidae, Chironomidae, Simuliidae, Oligochaeta and Ancylidae (Prat 2001).
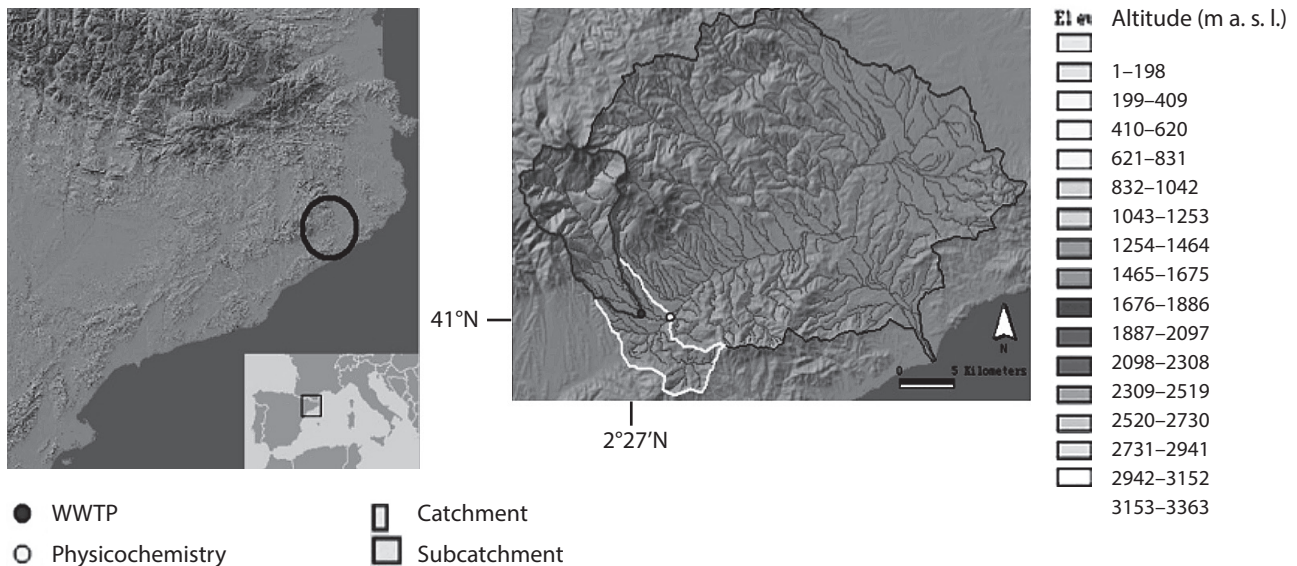
Fig. 1 Location of the La Tordera catchment area and the sub catchment (in grey) associated with the presence of the wastewater treatment plant. The area inside the white line is monitored by a physicochemical monitoring station.

Water samples were collected in triplicate from the hyporheic zone in the center of six transects located at intervals of 10 m along the two experimental sections during a hydrological year. Samples were collected using a drive-in metallic tube, 16 mm wide and 50 cm long, driven into the river bed to a depth of approximately 25 cm. A narrower tube of transparent plastic with a series of holes in the end pointing towards the river bed was then introduced inside the metal tube and interstitial water was pumped into it to a predetermined level (mini-well) using a peristaltic pump, discarding the first lot of excessively turbid samples. Once a volume of relatively transparent water was obtained, the temperature and specific conductivity (K25) of both it and the surface water adjacent to it, were determined. The samples were then immediately filtered through Whatman GF/F fiber glass filters, with a 0.7 µm pore size, and refrigerated until their analyzed in the laboratory. The dissolved reactive phosphorus was analyzed using a colorimetric method using molybdenum blue (APHA 1989) and the NO_3-N, NO_2-N and NH_4-N were analyzed automatically using a model II Technicon.

The Vertical Hydraulic Gradient (VHG) and Hydraulic Conductivity (K) were calculated for all transects in both sections. VHG was calculated by measuring the water levels inside the mini-well (piezometer) and the river surface, and then using the VHG equation to give both positive or negative values and thus the direction of surface/subsurface exchange. Hydraulic conductivity was calculated by filling the piezometer with river water using a plastic jug, and then measuring the time it took to empty to a certain level within the tube. This measurement was repeated three times and the data were then used in the respective equation.

The hyporheic fauna were collected from the intermediate zone of three transects (1, 2 and 3) in both experimental sections of the river in July. For this, a hydraulic metal tube with a mechanical water pump attached to the end of it was driven into the river bed to a depth of approximately 30–40 cm. Then, 50 l of hyporheic water was pumped into the tube through a 100 µm mesh sieve. The sieve was then washed in a plastic tray and the invertebrate sample collected placed in a plastic recipient where it was preserved and stained with rose Bengal. Samples were fully analyzed in the laboratory and organisms were identified to the taxonomic family level using the keys of Thorp and Covich (1991) and Merritt and Cummins (1978) which has been accepted as an excellent taxonomic category in most studies of biological indices of water quality (Barbour et al. 1992; Chesman 1995; Zamora and Alba-Tecedor 1996).

Formulas and Calculations

Vertical Hydraulic Gradient (VHG) (cm)

This parameter was obtained by dividing the difference between the water level inside the piezometer and the level of the river surface (Δl) between the depths of insertion of the piezometer in the sediment (h_{sed}) (Baxter et al. 2003):

$$VHG = \Delta l \, h_{sed}^{-1}$$

Hydraulic Conductivity (K) (cm s^{-1})

This parameter was calculated using Darcy's equation, which is based on the change in the position of the meniscus in the piezometer over a certain time and distance (Landon et al. 2001):

$$K = [L(t_1 - t_0)^{-1}] \ln[(H_0 \, H_1^{-1})]$$

where L is the segment of sediment under study and t is the time taken by the falling meniscus to cover the specified distance H. The data were analyzed using routine statistics (mean and variance). Paired t-tests were used to compare the means of the respective variables.

Data Analysis

All data were log-transformed prior to statistical analyses in order to stabilize variances and normalize the data sets. For the two sections studied we evaluated the effect of the effluent and the vertical hydraulic gradient (*VHG*) on the hyporheic habitat (nutrient concentrations, conductivity, temperature, hydraulic conductivity (*K*) and invertebrate assemblages) by comparing these parameters in the two sections sampled and upwelling – downwelling zones located upstream and downstream of the WWTP input using paired *t*-tests. The significance level used for all statistical tests was $p \leq 0.05$. The statistical analyses were performed using Sigma Plot 2000 for Windows statistical package. Hyporheic community parameters were used to compare invertebrate assemblages by higher groups (taxa richness (*S*), Shannon-Wiener diversity (*H'*), Jaccard's index) since there is evidence of a high correlation between the number of families and the number of species in different groups of organisms (Williams et al. 1997) and hyporheic BMWP index to compare water quality between the two sections. These analyses were performed using the statistical package InfoStat 1.2 for Windows, 2008. A between-groups principal component analysis (PCA) was used to assess spatial variation in hyporheic composition (Datry et al. 2007). Between-groups PCAs generate axes corresponding to the center of gravity for all the groups, which provides a representation of the spatial discrimination of sections. Taxa for which individuals comprised $\geq 2\%$ of the entire dataset were included in the analysis. Invertebrate abundances were $\log_{10}(x + 1)$ transformed prior to analysis. These analyses were carried out using InfoStat 1.2 for Windows.

Results

Comparison of Wastewater Treatment Plant Effluent and Stream Flow in Terms of Nutrient Loads

The values for the WWTP outflow were lower than for the discharge of the stream (0.02 and 0.065 up / 0.083 down m^3 s^{-1}, respectively) (Table 1). In La Tordera the WWTP effluent represented 100% of the stream discharge in August, September and October because the stream was completely dry upstream of the input. In contrast, the input only represented 3% of the stream flow when the stream discharge was at its highest. On average, the effluent made up 38% of the stream flow. Mean concentrations of NO$_3$-N and PO$_4$-P in the effluent were higher than in the stream (10.0, 3.0 and 4.11, 0.54 mg l^{-1} respectively). The QOD and BOD (35.0 and 14.0 mg O$_2$ l^{-1}, respectively) of the effluent indicated a significant input of organic matter into the stream (Table 1).

Hydrology and Chemistry of the Hyporheic Zone

There were successive zones in the vertical hydraulic gradients (VHG) of the upwelling and downwelling zones in both sections of the river (Fig. 2), with the mean values of the control being greater (0.192 and −0.101 cm cm^{-1}) than those of the altered section (0.101 and −0.096 cm cm^{-1}) (Table 2). There were no significant differences in VHG values for the six transects (spatial variation) or the two sections of the river ($p > 0.05$). The average value of the hydraulic conductivity (K) recorded in the altered section was lower than recorded in the control section (Fig. 3), but it could not be measured in three transects of the control section due to the high permeability of the substrate, which was composed mainly of gravel and rock. When these extreme values for the control were removed, no significant differences between the two sections of the river were recorded and K varied between 46.33 and 51.18 cm s^{-1}.

Table 1 Water temperature, hydromorphological features and indices of water quality recorded in La Tordera. Table compares data from upstream and downstream of the WWTP, with that of the WWTP effluent.

| Parameter	La Tordera		
	(upstream of WWTP)	WWTP effluent	(downstream of WWTP)
Temperature (°C)	17.9	–[b]	20.5
Width (m)[a]	6.75	–	6.33
Depth (m)[a]	0.09	–	0.11
Velocity (m s^{-1})[a]	0.13	–	0.14
Discharge (m^3 s^{-1})[a]	0.065	0.02	0.083
QOD (mg O$_2$ l^{-1})	–	35	–
BOD (mg O$_2$ l^{-1})	–	14	–
NO$_3$-N (mg N l^{-1})	2.71	10	2.57
PO$_4$-P (mg P l^{-1})	0.08	3	0.33

[a] According to Ortiz et al. 2006.
[b] No data available.

Table 2 Hydraulic and physicochemical characteristics of the water at the surface and the discharge (upwelling) and recharge (downwelling) zones in the two sections of the river studied. Mean values (+/− standard deviation).

Parameter	Control section (upstream of WWTP)		
	Surface	Upwelling	Downwelling
K (cm s⁻¹)	46.33 (35.27)		
VHG (cm cm⁻¹)		0.192 (0.192)	−0.101 (0.126)
Temperature (°C)	14.16 (3.96)	15.58 (4.15)	13.82 (3.78)
Conductivity (µS cm⁻¹)	155.74 (25.42)	157.43 (26.49)	162.21 (25.84)
PO4 (mgl l⁻¹)	0.08 (0.02)[a]	0.07 (0.06)	0.08 (0.06)
NO3 (mg l⁻¹)	2.71 (0.27)[a]	6.17 (2.32)	6.20 (2.7)
NO2 (mg l⁻¹)	0.21[a]	0.02 (0.01)	0.03 (0.04)
NH4 (mg l⁻¹)	0.28 (0.14)[a]	0.05 (0.02)	0.04 (0.01)
	Altered Section (downstream of WWTP)		
K (cm s⁻¹)	51.18 (43.41)		
VHG (cm cm⁻¹)		0.101 (0.079)	−0.096 (0.131)
Temperature (°C)	15.82 (3.99)	14.79 (4.35)	16.44 (3.82)
Conductivity (µS cm⁻¹)	215.0 (65.75)	215.48 (54.67)	208.08 (68.11)
PO4 (mg l⁻¹)	0.33 (0.12)[a]	0.37 (0.37)	0.57 (0.47)
NO3 (mg l⁻¹)	2.57 (0.3)[a]	11.44 (2.7)	9.5 (3.3)
NO2 (mg l⁻¹)	0.05[a]	0.1 (0.1)	0.1 (0.1)
NH4(mg l⁻¹)	1.59 (0.4)[a]	0.2 (0.2)	0.2 (0.2)

[a] According to Merseburger et al. 2005.
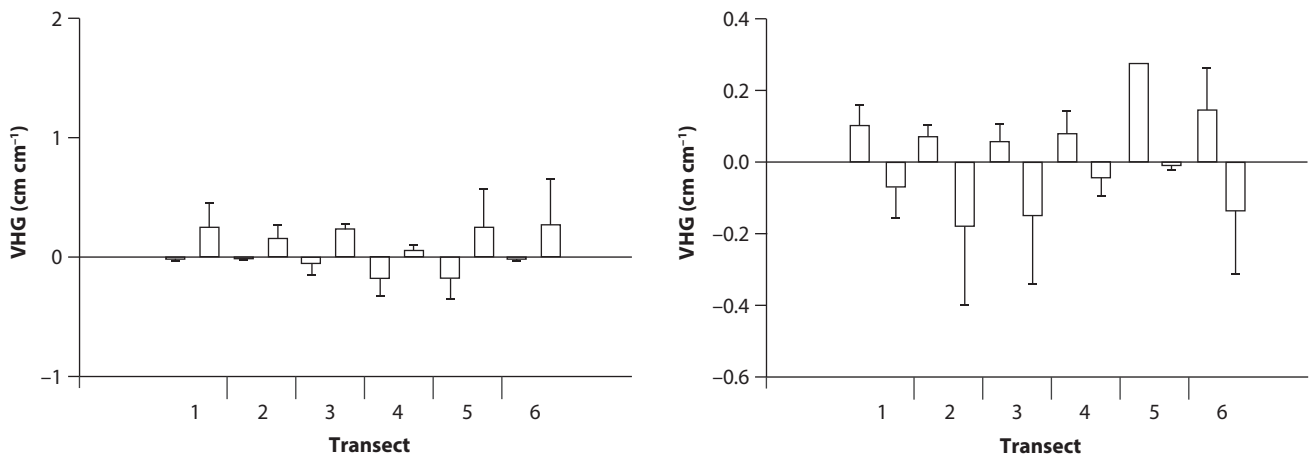
Fig. 2 Longitudinal variation in the hydraulic gradient (VHG) in up/down welling zones in the control (a) and altered (b) sections of the river.
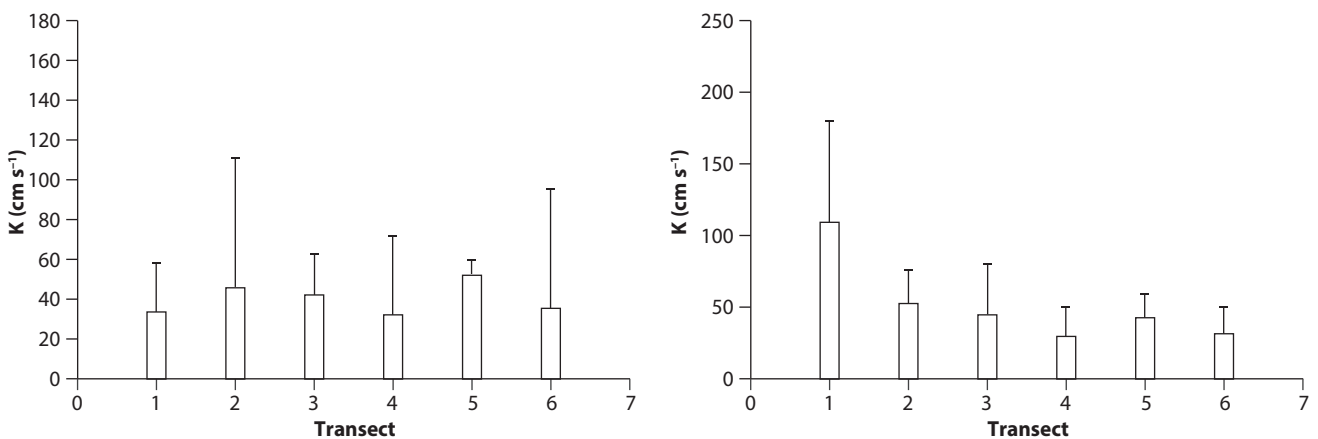
Fig. 3 Longitudinal variation in the hydraulic conductivity (K) recorded in the control (a) and altered (b) sections of the river.

The value of the specific conductivity was on average higher in the altered (215.0 μS cm^{-1}) than in the control section of the river (155.74 μS cm^{-1}). Similar conductivity means were recorded in upwelling and downwelling zones in both sections of the river probably because the values were very variable (Table 2). In general terms, there was a tendency towards higher conductivity values in the hyporheic zone compared to the surface water, especially in the control section of the river (Fig. 4). This tendency was not, however, significant ($p > 0.05$). On the other hand, a reduction in the conductivity in both sections was recorded as the warmer months approached. The water in the hyporheic zone was significantly warmer than that of the surface layer in the control section (paired t-test, $p < 0.05$) with a mean temperature of between 9.7 °C in January and 19.9 °C in July (Fig. 5). The altered section of the river was generally warmer than the control section with mean temperatures of between 10.5 °C in January and 21.2 °C in July. The hyporheic water in this section again tended to be warmer than that

of the surface layer, although these differences were not significant (Fig. 5).

Spatial Variation in Nutrient Concentrations

There were no significant differences in the mean concentrations of PO$_4$-P, NO$_3$-N and NH$_4$-N in the upwelling and downwelling zones in the control section of the river. In the altered section, however, the concentrations of NO$_3$-N in the upwelling zones were greater than in the downwelling zones, and the mean concentrations of PO$_4$-P were lower in the upwelling zones than the downwelling zones (Table 2). In all of the zones, the concentrations of NO$_3$-N and NH$_4$-N were generally more variable than the mean concentrations of PO$_4$-P. The interstitial water was richer in NO$_3$-N and NH$_4$-N than the surface water in the altered section of the river. During the course of this study the concentrations of NO$_3$-N in the interstitial water in the altered section of the river were close to 10 mg l^{-1}, three times greater than the concentrations of NO$_3$-N in the surface water. The hyporheic
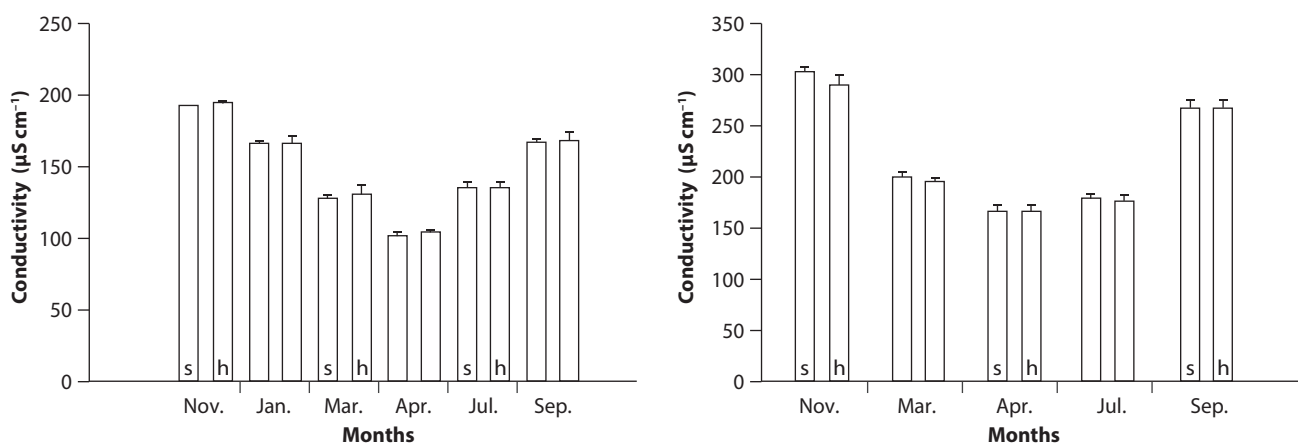

Fig. 4 Seasonal variation in the specific conductivity recorded at the surface and in the hyporheos in the control (a) and altered (b) sections of the river. Differences are not significant (s = surface, h = hyporheos).
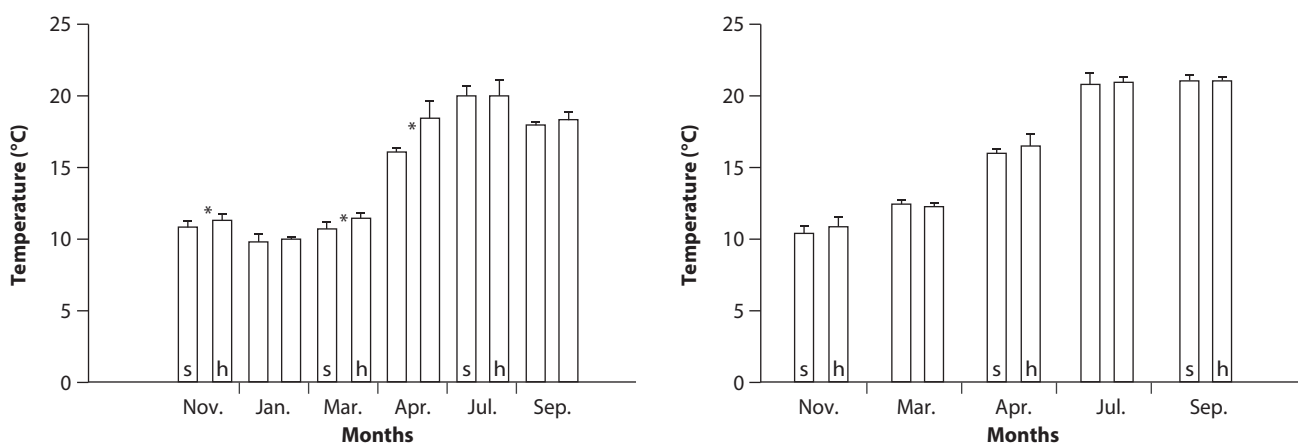

Fig. 5 Mean seasonal variation in the temperature recorded at the surface and in the hyporheos in the control (a) and altered (b) sections of the river (* = significant differences) (s = surface, h = hyporheos).
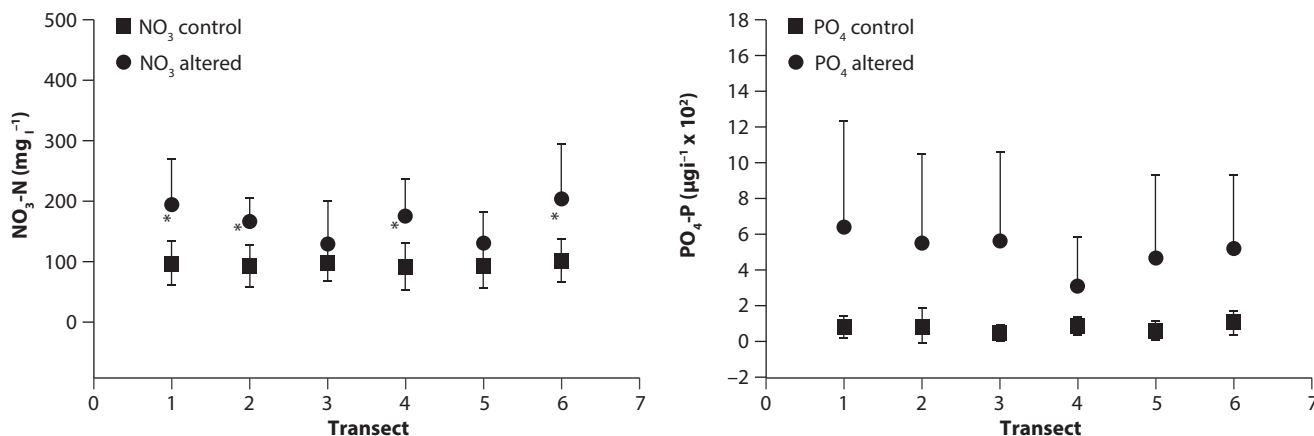
Fig. 6 Longitudinal variation in the mean hyporheic nitrate (a) and phosphate (b) concentrations recorded in the two sections river during the course of this study (* = significant differences).
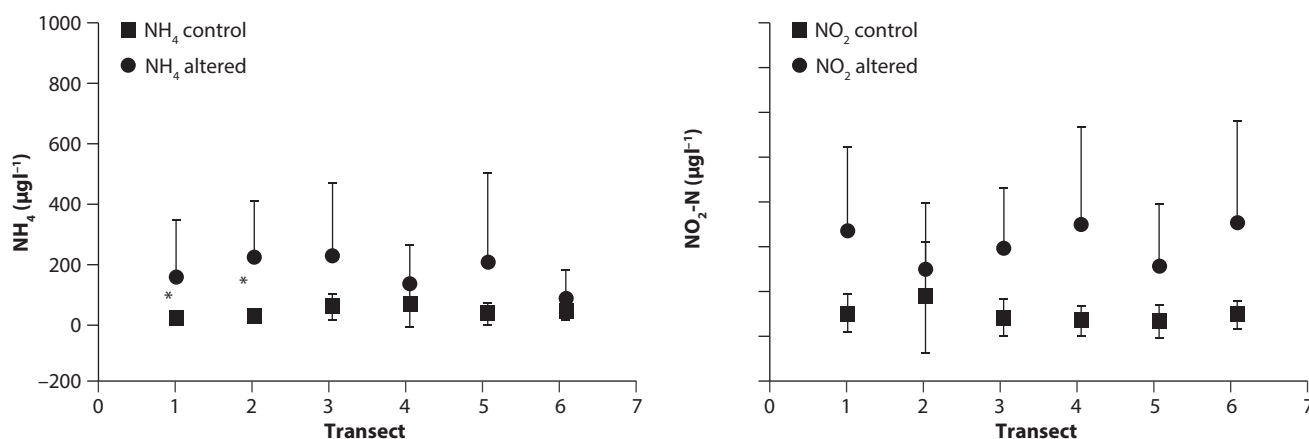

Fig. 7 Longitudinal variation in the mean hyporheic ammonium (a) and nitrite (b) concentrations recorded in the two sections during the course of this study (* = significant differences).

concentrations of NH_4-N were close to 45 and 200 μg l^{-1} for the control and altered sections, respectively, again much greater than those recorded for the surface water. The longitudinal variation in the interstitial concentrations of NO_3-N, PO_4-P and NO_2-N in the altered section of the river were similar, with a slight reduction along the lengths of the upper transects and a slight increase towards the lower transects (Figs. 6 and 7). In contrast, the pattern in the concentrations of interstitial ammonium was the reverse (Fig. 7), i.e., a tendency to increase along the upper transects and decrease towards the lower transects. In the control section of the river, there were no variations in the concentrations of these ions recorded along the longitudinal gradient. There were significant differences ($p < 0.05$, paired t-test) for nearly all of the comparisons of ionic concentrations in the two sections of the river.

Composition of the Meiofauna (*Hyporheos*)

A total of 24 taxa of hyporheic organisms caught in the three transects in each of the two sections of the river were identified. The most abundant groups of organisms were Oligochaeta, Chironomidae, Nematoda, Cyclopoida and Ephemerellidae (Table 3). The Dipterans were represented by four families: Chironomidae, Ceratopogonidae, Empididae and Simuliidae. The Ephemeropterans by two families: Ephemerellidae and Leptophlebiidae and the Syncarids by an extremely rare family: Parabathynellidae. Most of the Copepods belonged to the genus *Acanthocyclops* sp.

More groups of organisms were recorded in the control section of the river than the altered section with a maximum of 18 and a minimum of 12 taxa in the three transects studied. The lower transect (No. 6) in the altered section had the highest Shannon-Weaver diversity index (2.86), whilst in the upper transects the highest diversity index was recorded in the control section of the river, which is similar to the pattern recorded for group richness. Jaccard's index of similarity revealed a greater resemblance in community composition in the two sections in transect six (0.59) than in the other transects (0.50 and 0.36) (Table 3).

Table 3 Abundance of hyporheic organisms and community parameters recorded in each transect in the control and altered sections of the river.

Transect		Abundance range[a]					
		1 (upper)		3 (middle)		6 (lower)	
		Section					
Taxa		Contr.	Alter.	Contr.	Alter.	Contr.	Alter.
Platyhelminthes	Platy				1		2
Nematoda	Nema	3	3	3	3	3	3
Rotatoria	Rot	2	2	2	1		1
Tardigrada	Tard				1		
Oligochaeta	Olig	3	3	4	4	3	4
Hirudínea	Hir	1			2		
Gastropoda:							
Planorbidae	Plano	2					
Ostracoda.							
Candoninae	Cand	1	1		1	1	2
Cladocera:							
Alona sp.	Alo	2		3	1	2	2
Amphipoda:							
Niphargus sp.	Niph			2		2	
Copepoda:							
Cyclopoida:							
Acanthocyclops sp.	Acan	3	2	4	2	4	3
Harpacticoida:							
Bryocamptus sp.	Bryo	2		1		3	3
Syncarida:							
Parabathynellidae	Parab					3	
Acari	Acar	1	1	2		2	2
Collembola	Coll						3
Coleoptera:							
Haliplidae	Hali	1		3			
Diptera:							
Chironomidae	Chiro	4	4	4	3	4	4
Ceratopogonidae	Cera	1	1			2	
Empididae	Empi	1					
Simuliidae	Sim	1					
Ephemeroptera:							
Ephemerellidae	Eph	3	2	2		3	1
Leptophlebiidae	Lep		2	3	3	2	3
Heteroptera:							
Pleidae	Ple					1	
Plecoptera:							
Chloroperlidae	Chlo	1					
Community parameters							
Taxa richness (S)		18	10	12	11	14	13
BMWP [b]		46	29	35	28	37	34
Diversity index (H′)		2.59	2.23	2.54	2.29	2.45	2.86
Jaccard's index (contr. vs alter.)		0.50		0.36		0.59	

Table 3 Legend			
[a] Abundance of hyporheic organisms		**[b] Water quality in terms of the BMWP index**	
Range	**N° indiv.**	**Water quality**	**BMWP**
0	0	Pristine water	>100
1	1 to 3	Clean, but slightly polluted water	61–100
2	4 to 10	Polluted water	36–60
3	11 to 100	Very polluted water	16–35
4	> 100	Extremely polluted water	<15

The rarest or scarcest groups amongst the aquatic organisms in the hyporheic fauna were the Tardigrades collected in transect No. 3 in the altered section of the river; Gastropoda (Planorbidae) in transect No. 1 in the control section; Syncarids (Parabathynellidae) in transect No. 6 in the control section; Collembola in transect No. 6 in the altered section; Diptera (Empididae and Simuliidae) in transect No. 1 in the control section and Heteroptera (Pleidae) in transect No. 6 in the control section.

In terms of the BMWP (Biological Monitoring Working Party) biological index for water quality, the control section of the river had the highest values (Table 3) with transect No. 1 having the highest index (46). The lowest values were recorded in transect No. 1 in the altered section of the river with an index of 29 and transect No. 3 with an index of 28. According to the BMWP scoring system for the evaluation of water quality the control and altered sections of the La Tordera River are classified as *polluted* and *heavily polluted,* respectively. The decrease in water quality down river of the discharge point of the sewage plant was determined on the basis of the absence of the following families of aquatic insects: Ceratopogonidae, Empididae and Haliplidae.

The highest Shannon Weaver diversity index recorded in transect No. 6 in the altered section of the river (2.86) corresponded to the highest values of the Vertical Hydraulic Gradient (0.273 cm cm^{-1}), specific conductivity (185.2 µS cm^{-1}), temperature (22.2 °C), PO$_4$-P (5.35 × 10^2 µg l^{-1}) and NO$_3$-N (23.3 mg l^{-1}). Nevertheless,

transect No. 1 in the control section of the river had the second highest diversity index (2.59) and was the highest in hyporheic organism richness (18 groups). In contrast to transect No. 6 in the altered section, however, these values corresponded to the lowest for PO$_4$-P concentrations (0.9 × 10^2 µg l^{-1}), NO$_3$-N (12.63 mg l^{-1}), NH$_4$-N (0.3 µg l^{-1}) and NO$_2$-N (70.52 µg l^{-1}). It is important to note that the values given in Table 3 are the results for only one sample collected in July, which precludes more consistent and robust correlations. Thus, we can only speak in terms of tendencies and a preliminary characterization of the meiofauna in these two sections of the river. In general, the altered section of the river had higher nutrient concentrations and lower biological indices of water quality than the control section.

Ordination Analyses

The between-sections PCA was significant ($p = 0.019$) and indicated that 99% of the total variance in the taxonomic data was explained by spatial variation (Fig. 8) (axis 1 = 74.2%; axis 2 = 25.8%). Axis 1 was best explained by a positive relationship with Chironomidae, Oligochaeta, Nematoda, *Acanthocyclops* sp.*,* Leptophhebiidae, *Bryocamptus* sp. and *Alona* sp. and a negative relationship with Planorbidae and Rotifera. Axis 2 was positively related to Platyhelminthes, Tardigrada and Candoninae, and negatively to Parabathyneliidae and *Acanthocyclops* sp. There was a clear separation between taxa recorded in the control section (Parabathyneliidae and *Acanthocyclops*) and those recorded in the altered section (Chironomidae, Oligochaeta, Nematoda).
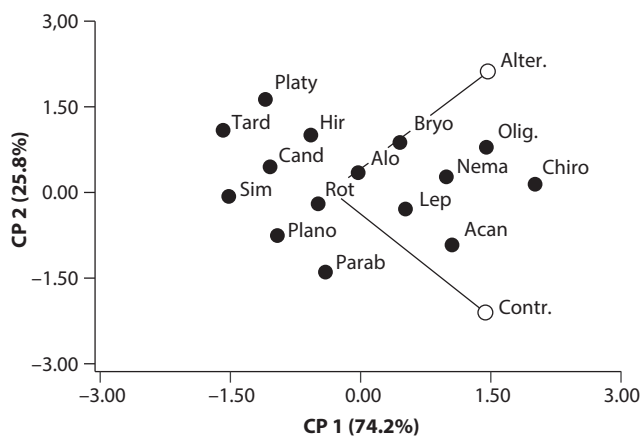
Discussion

Surface – Hyporheos Interactions

The exchange processes between the surface and the hyporheos in the La Tordera River are clearly related to the spatial-temporal variations in the vertical hydraulic gradient and hydraulic conductivity. The spatial alternation of upwelling and downwelling zones in both sections of the river indicate the geomorphology is similar in both sections of the river; the river bed is mainly made up of coarse sediments (pebbles, stones, gravel) resulting in high values of hydraulic conductivity, especially in the control section. The changes recorded in the magnitude and direction of the hydraulic gradient could be the re-

Fig. 8 Between-groups principal component analysis (PCA) of hyporheic meiofauna using log(x + 1) transformed data (Alter = altered section; Contr. = control section).

sult of changes in water levels and surface water discharge (Valett et al. 1994). An increase in the surface water discharge could increase the extent of the upwelling zones, whilst in dry periods the downwelling zones would increase in size (Valett et al. 1990).

The importance of the topography of the river bed in hydrological exchange processes has been demonstrated for longitudinal sections along riffle – pool sequences. Reductions in depth result in high pressure zones at the end of a pool where surface water infiltrates the sediment, displacing the interstitial water (downwelling). Later, at the extreme end of the riffle, the increase in depth results in a low pressure zone, causing the discharge or upwelling of interstitial water (Triska et al. 1989b; Brunke and Gonser 1997; Woessner 2000). Experiments done with tracers by Harvey and Bencala (1993) indicate that these convex and concave sequences along the topography of the river bed control surface – hyporheic interactions. Surface water infiltrates in downwelling zones and flows interstitially along well defined channels. After travelling up to 10 m downstream it returns to the surface independently of contributions from the river aquifer. The obstacles present on the river bed, such as gravel bars, rocks, pebbles, tree trunks act as dykes, and aggregations of macrophytes and even lamprey nests, can result in small scale differences in hydraulic pressure modifying interstitial water flow (Hendricks and White 1991). The lack of important topographic differences in the two sections of the La Tordera River used in this study could be the reason why the hydraulic gradient does not show a defined pattern of upwelling and downwelling zones. In contrast, the higher hydraulic conductivity recorded in the control section of the river compared to the altered section demonstrates the effect of the effluent from the sewage treatment plant. This point source of pollution results in the clogging of the hyporheic interstitial spaces causing a clear reduction in the hydraulic conductivity in the altered section of the river. The discharge of residual water, or water inefficiently or improperly treated encourages the external clogging of the river bed by favouring the growth of benthic algae or the sedimentation of fine or very fine organic particles on the river bed. Internal clogging is caused by the intrusion of organic or very fine inorganic material into the interstitial spaces in the sediment. Unsustainable activities as regards soil use in the catchment basin directly enhance this process by increasing the amount of suspended particles (seston) and sediment load (Brunke and Gonser 1997; Treese et al. 2009).

The small difference in the specific conductivity of the surface water and the water in the hyporheos zone is similar to that reported by other authors such as Hendricks and White (1991) for the Michigan River, where the hyporheic conductance was significantly higher than that of the surface water. Although the specific conductance of the hyporheos also tended to be higher than that of the surface water in this study, the differences were not significant due to the large variability between replicates.

Surface – aquifer exchange processes determine the temperature in the hyporheic zone in that the hyporheic temperature in general, varies according to the mean annual air temperature, whilst that of the river shows large daily and seasonal fluctuations. The temperatures recorded in this study clearly tended to be higher in the hyporheic zone than at the surface and these differences were significant in the control section of the river. These results agree with those reported by Valett et al. (1990) and Valett (1993) for rivers in the Sonora Desert with highly porous sediments. The temperature regimen in the hyporheic zone is important for both surface and underground aquatic systems due to the fact that fluvial microbial metabolic processes and the development of many invertebrates are thermo-dependant (Brunke and Gonser 1997).

Hydrology and Nutrient Content

The hyporheic concentrations of total inorganic nitrogen (NH_4-N + NO_3-N + NO_2-N) and phosphate (PO_4-P) were higher than those recorded at the surface. There were no significant differences between upwelling and downwelling zones. A large number of publications report the richness of the nutrients in the hyporheic zone (Valett et al. 1990, 1994 and 1996; Stanley and Boulton 1995; Jones Jr and Holmes 1996). There is a strong correlation between surface nitrate concentrations and the magnitude of the vertical hydraulic gradient (VHG) in upwelling zones. The results from this study indicate that contributions from nitrogen-rich interstitial waters may increase surface nitrate concentrations in upwelling zones, thus making the hyporheic zone a source of nitrates in the control section of the La Tordera River. In contrast, in the altered section of the river there is a high concentration of surface NO_3-N, NH_4-N, DIN and DOC as a consequence of the discharge from the wastewater treatment plant (Merseburger et al. 2005). Similar studies indicate that in these situations the hyporheic zone acts as a nutrient sink or trap (Triska et al. 1989; Jones Jr and Holmes 1996; Taleb et al. 2008). In the hyporheic zone, especially in the aerobic – anaerobic interphase, an intense metabolism of nitrogen takes place during the nitrification – denitrification processes. Both processes are related to the existing gradients of dissolved oxygen, dissolved organic carbon (DOC), ammonium and nitrates as well as flows between the surface and aquifer compartments. Allochthonous sources of nitrate entering the river may be assimilated by the epiphyton, which stimulates the growth of benthic algae. This constitutes one of the most important effects of point sources of pollution by nutrients in oligotrophic water bodies (Valett et al. 1992; Jones Jr and Holmes 1996). There were no significant differences in the phosphate concentrations between the upwelling and downwelling zones in most of the samples, although there was a tendency for them to be higher in the downwelling zone in the altered section of the river. Valett et al. (1994) report similar results for Sycamore Creek, Arizona and also establish that the

hyporheic phosphate concentrations are not affected by the magnitude of the surface – aquifer exchange in the upwelling zones, although they were very close to being significant. The high concentrations of phosphate recorded in the hyporheic zone and their relation to the VHG indicate that the dynamics of this anion are controlled by physicochemical processes such as adsorption. Biological processes may also affect the concentrations of this anion, but in this study area are probably insignificant due to the fact that there is an excess of this nutrient, especially in the altered section of the river (Valett et al. 1990).

Composition of the Meiofauna and its Relation to Hyporheic Physicochemistry

Chironomids were the most abundant taxon in the hyporheos in the area studied, followed by Oligochaetes. These results are similar to those reported by Fraser and Williams (1998) and Franken et al. (2001) in Ontario, Canada. The number of taxa identified by these authors was also similar to that recorded in this study. Nevertheless, Rotifers, Cladocera and Syncarids recorded in the La Tordera River in this study have not been reported previously in this region (Boulton and Stanley 1995; Fraser and Williams 1998; Bartoszek 2001; Danielopol and Pospisil 2001; Franken et al. 2001). The Rotifera and Cladocera recorded in the hyporheic samples form part of the fluvial benthos, which indicates that these organisms also occasionally visit the interstitial habitat in search of food or refuge (Williams 1993). Amongst the groups identified, the Syncarids are rare and a most important find.

The multivariate analysis indicated that the species composition of the invertebrates recorded in the two sections differed. We suggest that this reflects differences in the taxa in their tolerance of pollution. Species richness and diversity did not vary greatly, possibly reflecting the rapidity with which most hyporheic sensitive taxa, such as Cyclopoida, Syncarids, Ephemeroptera and Gastropoda are replaced. The Amphipoda, Syncarida, Coleoptera, Heteroptera and Gastropoda were absent from the altered section. Belaidi et al. 2004 report that Crustacea do not occur in heavily polluted subterranean water in the Tafna stream in Algeria, which demonstrates the vulnerability of these organism to pollution and their value as indicator organism in subterranean environments. The highest richness of meiofauna was recorded in the control section of the river, where the interstitial nutrient concentrations were lowest. The meiofauna richness recorded in the aquifer discharge (upwelling) zones in all transects in both sections of the river was high. This distribution can be attributed to several factors: habitat differences, response to changes in sediment size and underflow dynamics, and to increasing hypoxia and pollution (Belaidi et al. 2004). Franken et al. 2001 and Febria et al. 2010 report a large number of groups of organisms in aquifer recharge (downwelling) zones as well as in upwelling zones. As regards the preferences of a group of organisms for a particular section of a hydraulic gradient, the results of this study are not conclusive owing to the lack of samples of the hyporheic fauna in downwelling zones.

In an attempt to determine the quality of the interstitial water in the two sections of the La Tordera River we used the BMWP (Biological Monitoring Working Party) biological index specially adapted for Mediterranean rivers (Armitage 1983; Alba-Tercedor and Sánchez-Ortega 1988), which takes into consideration the presence/absence of aquatic benthic invertebrates in the hyporheic zone. We considered it appropriate to apply this index to the hyporheos in this study because many benthic larvae occupy the hyporheic zone during part of their lifecycle, as well as those groups that permanently occupy this habitat. According to the BMWP index the control section of the river is *polluted* and the altered section *heavily polluted*. The development and application of a standardized sampling technique in this study is analogous to the establishment of sampling methods for assessing the biological quality of rivers using the BMWP/ASPT scoring system (Hawkes 1997). Although the range of BMWP scores generated from the samples collected is roughly comparable to that expected from standard benthic kick sampling, the calculated scores for the hyporheic zone are much lower. The BMWP scoring system was designed to highlight biological differences in a wide spectrum of different types of river (from high altitude torrential streams dominated by Ephemeroptera, Plecoptera and Trichoptera to sluggish, lowland rivers dominated by Chironomids and Oligochaetes). In contrast, the hyporheic zone, although not actually a natural part of that spectrum, is analogous to a highly truncated portion of it, with all samples being taken from essentially the same microhabitat, almost regardless of the character of the overlying river. The range of back-calculated values for benthic BMWP (121–171) (Prat et al. 2001) is thus much narrower than the hyporheic BMWP values recorded in this study (28–46). Within the hyporheic zone samples, therefore, it is much more likely that high BMWP scoring taxa will occur alongside low scoring taxa than would be the case in the benthos.

When the taxa richness of the benthic samples (Ortiz et al. 2006) is analyzed the BMWP index is much higher than that recorded for the hyporheic samples. This reflects the relative scarcity of benthic macroinvertebrates in the hyporheic zone, the truncated functional biodiversity for these organisms and also the dominance of meiofauna in the sample that was not collected using a standard benthos net (the mesh being too coarse). When assessing the conservation value of a site the taxonomic richness of the fauna is one of the primary measures used (Pryce et al. 2010). Of the additional (non-BMWP) taxa recovered using hyporheic techniques the majority were ubiquitous (e.g. Nematoda, Cyclopoida and Harpacticoida Copepoda, Rotatoria) (Table 3). Other groups were present in very low numbers (Tardigrada, Planorbidae, Empididae were collected in only one section). Six taxa are potential bioindicators (present in most sections): Cladocera,

Ostracoda, Ceratopogonidae, Acari, Platyhelminhes, Hirudinea. However, little is known about their relative tolerance of pollution and disturbance and more research would be required before they could be used as bioindicators. It is also possible that they are routinely present in kick samples but either pass through the mesh or are simply too small to be recorded in the normal sample sorting procedure. Hyporheic assemblages are located at the interface between ground and surface waters and are therefore able to give an earlier warning of the ecological response of lotic ecosystems to groundwater borne pollution. Groundwater species provide the potential for biomonitoring aquifers and assessing their response to pollution (e.g. diffuse nitrogen pollution). Monitoring of hyporheic and groundwater assemblages would provide information on the health of these compartments of the lotic ecosystem and provide a more comprehensive assessment of a river's ecological status (Robertson et al. 2008). This monitoring would be particularly timely in the light of the Groundwater Directive 2006, which encourages member states to conduct research in order to provide better criteria for ensuring groundwater ecosystem quality and protection.

The effluent from the Santa María de Palautordera wastewater treatment plant contributes a high nutrient load. When N induces a change in the dominant form of transport (NO_3-N) to the reduced form (NH_4-N), this has a negative effect on stream biota, especially fish communities (Marti et al. 2004). The WWTP effluent is an important source of contamination resulting directly or indirectly in external clogging of the interstitial spaces in the sediment, reducing the available physical space for the development of the hyporheic fauna and producing an increase in benthic algal cover. The fauna in the interstitial biotope appears to be highly dependent on sediment porosity and composition. The presence of a potentially highly biodegradable biomass favours detritivors and microbial organisms over filter feeders (Table 3), resulting in a change in the composition and structure of the hyporheos (Brunke and Gonser 1997; Palmer et al. 1997; Stepenuck et al. 2002; Morais et al. 2004). In general, this community is extremely sensitive to external perturbations, and is categorized as having *low resistance* (Fowler and Death 2001; Sliva and Williams 2005). In addition, subterranean, lenthic and oligotrophic lotic ecosystems have a long recovery time, that is, *low resilience* (Stepenuck et al. 2002; Febria et al. 2009). Thus, these perturbations, principally of anthropic origin, are damaging both to the ecological equilibrium and the autoregenerating capacity of the hyporheic zone, the main link in the process of integral recovery of fluvial ecosystems.

Acknowledgements

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