

POINT SOURCE EFFECTS ON DENSITY, BIOMASS AND DIVERSITY OF BENTHIC MACROINVERTEBRATES IN A MEDITERRANEAN STREAM

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ABSTRACT

We sampled benthic macroinvertebrates above and below a point source effluent in La Tordera stream (NE, Spain) over 2001–2002 to assess the effects of nutrient enrichment on the structure, and taxonomic composition of the benthic macroinvertebrate community. Below the point source, discharge, specific conductance and nutrient concentrations were higher than at the upstream reach, while dissolved oxygen (DO) decreased. Macroinvertebrate density was higher at the downstream reach than at the upstream reach on most dates but the two reaches did not differ in macroinvertebrate biomass.

On average, taxa richness at the upstream reach was 20% higher than at the downstream reach. Several taxa, especially mayflies, stoneflies and caddisflies, were present only at the upstream reach. Shannon diversity was similar between the two reaches on 50% of the dates. Ordination analysis clearly separated the samples of the upstream reach from the samples of the downstream reach in the first axis and corroborated the effect of the point source on the benthic community. The two reaches followed a similar temporal pattern with respect to the distribution of taxa along the second axis of the ordination analysis. Higher similarities between the two reaches in taxa composition, densities and biomass after the spates of April and May 2002, suggest that flooding events may act as a reset mechanism for benthic communities and play an important role in stream restoration. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS: wastewater treatment plant; benthic macroinvertebrates; flooding; nutrient enrichment; disturbance

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INTRODUCTION

Human activity has long been known to have dramatic effects on stream invertebrates (Hynes, 1978; Wiederholm, 1984; Rosenberg and Resh, 1993; Suren, 2000). Among other types of human disturbances to stream ecosystems, nutrient enrichment decrease macroinvertebrate richness (Paul and Meyer, 2001) by elimination of sensitive taxa, mostly represented by the insect orders Ephemeroptera, Plecoptera and Trichoptera (EPT; Lenat, 1983). Simultaneously, taxa considered resistant to pollution and adapted to unstable habitats, such as midges and oligochaetes, are enhanced (Hynes, 1978). This response to nutrient enrichment is well established worldwide, in arctic (Milner and Oswood, 2000; Benstead *et al.*, 2005), tropical (Ometo *et al.*, 2000; Thorne *et al.*, 2000), desert (Dor *et al.*, 1976; Voelz *et al.*, 2005), temperate (Roy *et al.*, 2003) and Mediterranean streams (Prat *et al.*, 1984; Prenda and Gallardo-Mayenco, 1996). However, the response of other community parameters, such as macroinvertebrate density, to point source effluents is not consistent throughout. Several studies found a decrease in macroinvertebrate density while others noticed an increase. Paul and Meyer (2001) attributed such incongruence to the separate effects of toxic compounds and nutrient enrichment, respectively. However, high nutrient concentrations can also result in a decreased density (Hynes, 1978). Much less is known about the effects of nutrient enrichment on macroinvertebrate biomass. Wallace and Webster (1996) argued that when resource availability is high, invertebrates have to invest less energy in searching for food resulting in an increase of biomass. Although not

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difficult to test, few studies have examined changes in macroinvertebrate biomass due to nutrient enrichment. Macroinvertebrate biomass is not commonly used in bioassessments because of the effort to obtain good estimates (Bernardini *et al.*, 2000) and the lack of studies to obtain clear patterns in response to organic pollution.

Benthic macroinvertebrates are widely used for bioassessments, but temporal variation of the benthic community is rarely considered (however, see Sandin and Johnson, 2000; Morais *et al.*, 2004). In this sense, unrepeated assessments may lead to misguided conclusions because natural disturbance and population dynamics are susceptible to significantly alter the community structure. Moreover, little attention has been paid to flow disturbance in polluted streams, even though it is known to play a central role in determining the structure of stream communities (Lake, 2000).

We sampled the benthic macroinvertebrate community on six sampling dates over the hydrologic year 2001–2002 in one reach upstream and one reach downstream of a point source in La Tordera stream (Catalonia, NE Spain). Our objectives were to: (1) assess the effect of the point source on the structure (density, biomass and diversity) and community composition of the benthic macroinvertebrate community, (2) determine which environmental and point source variables best explain the differences between the two reaches in terms of macroinvertebrate density, biomass, diversity and community composition, and (3) examine the temporal variability of the considered community descriptors over a hydrologic year. We hypothesized that density and biomass of macroinvertebrates would increase below the point source according to previous studies conducted in stream ecosystems under moderate nutrient enrichment. We also expected a decrease of taxa richness and an increase of dominance by tolerant taxa below the point source that would translate into a lower diversity and community structure.

STUDY SITE

The study was conducted in La Tordera stream in Catalonia (NE Spain; Figure 1). We selected one reach upstream and one reach downstream of the input of the wastewater treatment plant (WWTP) of Sta. Maria de Palautordera to examine the effects of this point source and related outflows on several descriptors of the benthic macroinvertebrate community.

At the sampling site (41°41' N, 2°27' E, 200 m a. s. l.), La Tordera is a third-order stream draining a catchment of 80 km² dominated by a sclerophyllous forest of several species of *Quercus* (Figure 1). Small patches of irrigated crops are present in the lower part of the catchment, surrounding the urban area. The geology of the catchment is mainly siliceous, dominated by slates and phyllites. The climate is Mediterranean, with mean air temperatures ranging from 5 (January) to 23°C (August). Mean annual precipitation is 575 mm, which mostly occurs in spring and fall. Stream discharge is highly variable within and among years. In dry years, most sections of the stream became isolated pools in the summer, but below the WWTP input the effluent outflow was sufficient to maintain continuous flow for hundreds of metres of stream channel. Spates are associated with rainstorms and usually occur in spring and autumn. Mean annual discharge of the stream during the hydrologic year 2001–2002 (0.20 m³/s) was three times lower than the mean discharge for the period comprised between 1923 and 2002 (0.77 m³/s) and was preceded by 4 years of similar mean annual discharge (0.27 m³/s; Figure 2a). However, during the sampling period discharge peaked in spring while during the previous 4 years the maximum occurred in winter. The upstream reach was dry from July to early October 2001. The two reaches were affected by spates in the middle of November 2001, and early March, April, May and June of 2002 (Figure 2b). The most significant spate during the sampling period was in May, when stream discharge was over 7 m³/s.

The population of the catchment in 2001 was 8564 inhabitants, 93% of which were concentrated in the lower part of the catchment, in the villages of Sta. Maria de Palautordera and St. Esteve de Palautordera. The mean outflow of the WWTP effluent to La Tordera stream is 1300 m³/day, while the discharge is highly variable both daily and seasonally. The WWTP performs a biological treatment with activated sludge, but lacks the technology to actively remove nitrogen or phosphorus. Diffuse sources from small-scale agriculture and small tributaries also affect La Tordera stream, but they are located near the WWTP effluent input and were considered together as a point source for the downstream reach. The point source considerably increased nutrient concentrations in La Tordera stream and persisted several hundred metres below. Any tributary joins the stream between the WWTP input and the downstream reach and there were no diffuse sources under dry conditions (Merseburger *et al.*, 2005).

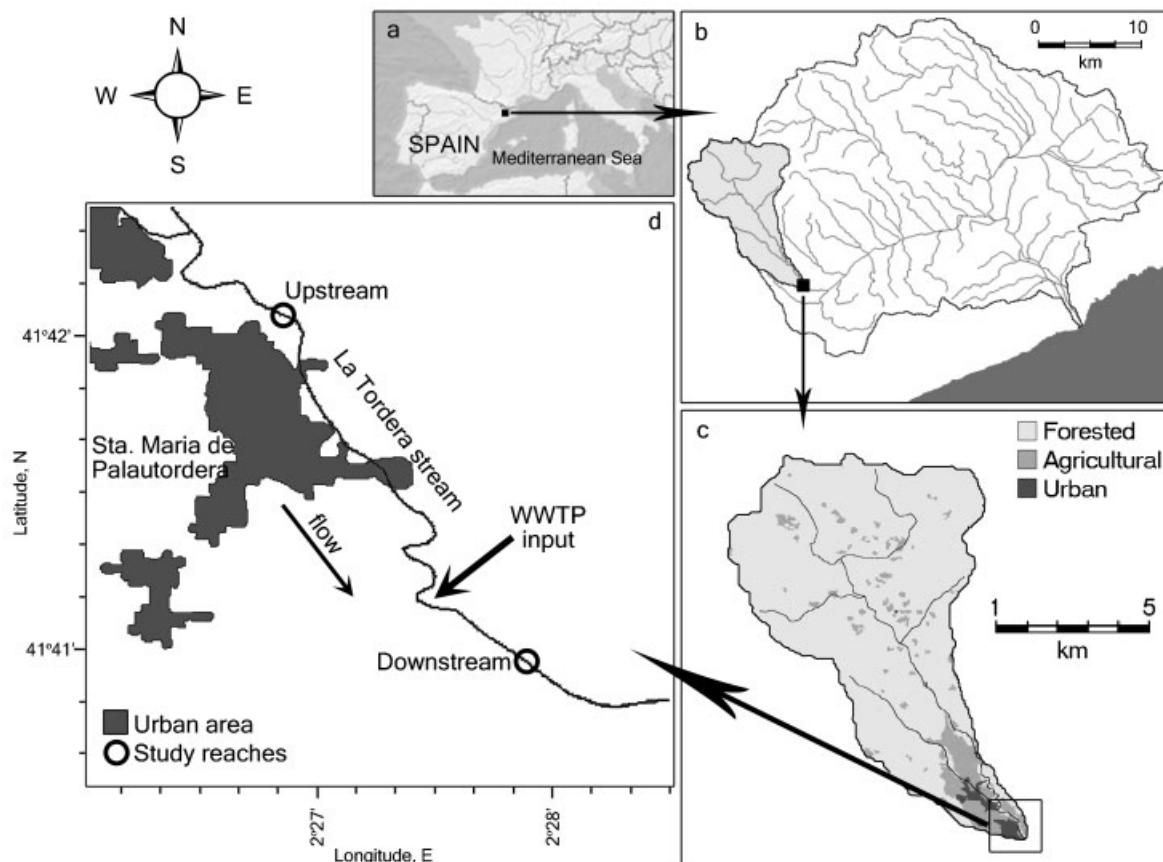


Figure 1. Location of La Tordera stream in Catalonia, NE Spain (a) La Tordera catchment and the subcatchment affecting the sampling site, highlighted in grey (b) land uses of the subcatchment affecting the study reaches (c) and location of the study reaches in relation to the wastewater treatment plant (WWTP) input (d) data from the Catalan Cartographic Institute (ICC)

METHODS

Experimental setting

We selected two 100 m long run-riffle reaches with similar substrata type and canopy cover located upstream and downstream of the WWTP effluent input. The upstream reach was located 3 km above the WWTP effluent input and served as a reference reach. The downstream reach was located 500 m below the WWTP effluent input. We collected samples on six dates over the hydrologic year 2001–2002: November of 2001 and January, March, April, June and September of 2002.

Physical and chemical parameters

In each reach and sampling date, we measured velocity using a Neurtek Instruments[®] Miniair 2 flow metre, depths and width in six predefined transects to estimate discharge according to the velocity-area method described in Gordon *et al.* (1992). Water temperature and dissolved oxygen (DO) concentration were measured in each reach over 24 h cycles on each sampling date with a WTW[®] Oxi 340-A oxygen metre. We measured water conductivity using a WTW[®] LF 340 conductivity metre.

Water samples for nutrient analysis and dissolved organic carbon (DOC) were taken in each reach, filtered on site through preashed Whatman[®] GF/F glass fibre filters and stored on ice. Ammonium (NH_4^+ -N) concentration was analysed on a Bran-Luebbe[®] Technicon Auto analyzer II. Nitrite (NO_2^- -N), nitrate (NO_3^- -N), and soluble reactive

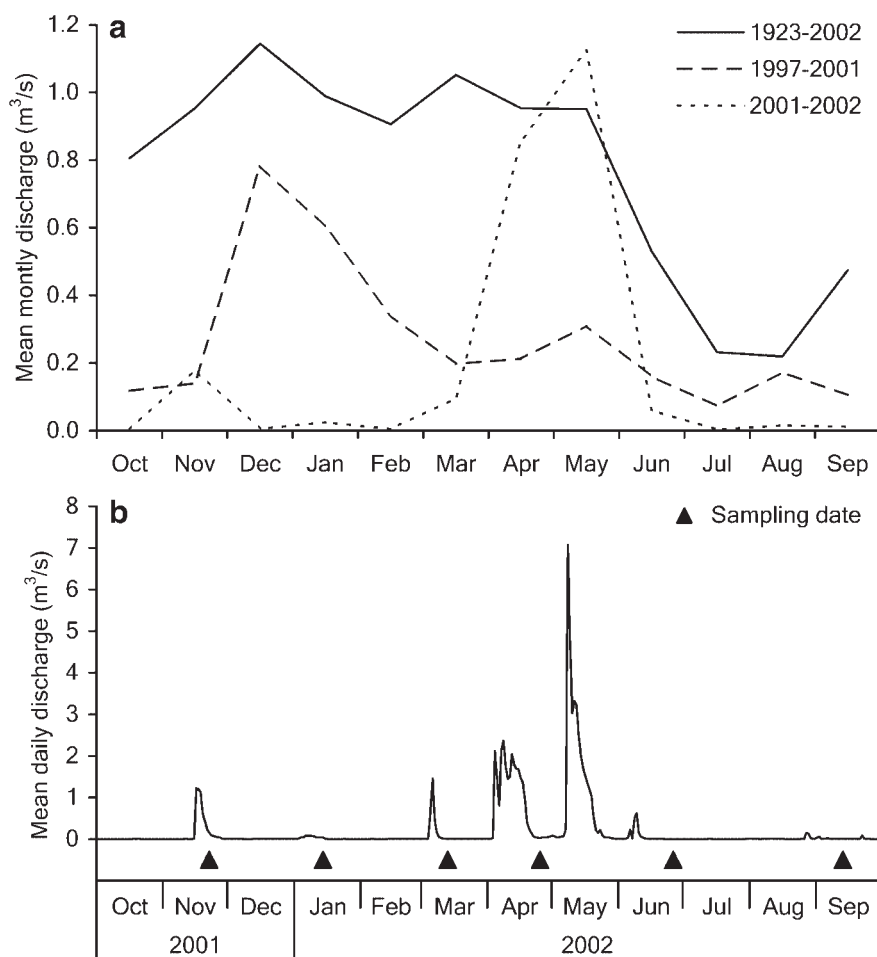


Figure 2. Mean monthly discharges (m^3/s) of La Tordera stream over the periods 1923–2002, 1997–2001, and 2001–2002 (a) and mean daily discharge during the sampling period (2001–2002); (b) continuously measured 2 km below the downstream reach. The arrowheads indicate sampling dates. Data from a gauging station maintained by the Catalan Water Agency (ACA)

phosphorus (SRP) concentrations were analysed on a Bran-Luebbe[®] TRAACS 2000 Auto analyzer. NO_2^- -N and NO_3^- -N were analysed using the cadmium-copper reduction method, and SRP was done using the molybdenum blue colorimetric method. Dissolved inorganic nitrogen (DIN) was calculated by summing the concentrations of NH_4^+ -N, NO_2^- -N, and NO_3^- -N. DOC concentration was analysed using high-temperature catalytic oxidation (Shimadzu[®] TOC 5000 analyzer).

Benthic macroinvertebrates

Six modified Surber sample (625 cm^2 , $250 \mu\text{m}$ mesh size) were taken in each reach and sampling date. Samples were preserved in the field with 4% formaldehyde solution or frozen. In the laboratory, heavier inorganic substrates were removed by elutriation. All large invertebrates ($>5 \text{ mm}$) were hand-picked from the samples and then preserved in 70% ethanol. If necessary, the smallest invertebrates ($250 \mu\text{m}$ – 5 mm) were subsampled on an area basis (Moulton *et al.*, 2000). Invertebrates were counted by handpicking with the aid of a dissecting microscope at

15× magnification, identified to the lowest practical taxonomic level, enumerated, dried (60°C until constant weight) and weighed to obtain dry mass.

Data analysis

We calculated several macroinvertebrate community descriptors for each reach and sampling date, including density (individuals/m²), biomass (g DW/m²), taxa richness (S), rarefaction coefficient (ES₁₀₀), as number of taxa expected for 100 individuals (Heck *et al.*, 1975; Walsh *et al.*, 2001), Shannon–Wiener diversity (H'; Lloyd *et al.*, 1968), Pielou's evenness (J'; Clarke and Warwick, 1994), and Ephemeroptera, Plecoptera and Trichoptera (EPT) richness (Lenat, 1983; Barbour *et al.*, 1999). Taxa richness and EPT richness were calculated from taxa present in each reach and sampling date. Rarefaction coefficient, Shannon diversity and Pielou's evenness were calculated from averaged macroinvertebrate densities of each reach and sampling date using the DIVERSE analysis routine of the computer package PRIMER (for Windows, version 5.2.2, Plymouth Marine Labs, Plymouth, UK).

We contrasted physical and chemical variables, between the two study reaches over the sampling period through paired *t*-test. Macroinvertebrate density and biomass were compared between stream reaches using one-way analysis of variance (ANOVA) procedures. Pairwise comparisons among group means were made using Tukey's studentized range test (HSD). Reach taxa richness, rarefaction coefficient, Shannon–Wiener diversity, Pielou's evenness and EPT richness were compared by using paired *t*-test. Data were either log₁₀ (*x* + 1) or arcsin-square root transformed prior to analysis when necessary to meet assumptions of normality and homoscedasticity.

We correlated the considered community descriptors to the effect of the relative contribution of the point source and environmental conditions using two-tailed Spearman's rank correlation (*R*_S) to assess which variables best explain the differences between the two reaches. The relative contribution of the point source was determined in terms of discharge and concentrations of DIN and SRP and was calculated as the difference between the value at the downstream reach and that at the upstream reach standardized for the value at the upstream reach in each sampling date. The considered environmental conditions were mean water temperature and peak discharge during the previous spate to each sampling date. The response of each community descriptor was measured as the difference between the value at the upstream reach and that at the downstream reach in each sampling date. We determined the weight of Pielou's evenness and taxa richness on Shannon diversity by using two-tailed Pearson's correlation (*r*). These analyses were performed using the statistical package SPSS (for Windows, version 11.0.1, SPSS Inc., Chicago, Illinois).

We used ordination analysis to explore taxa and sample distribution within reaches and sampling dates. Initial analysis using detrended correspondence analysis (DCA) revealed that the macroinvertebrate data set had a gradient length shorter than two standard deviation (SD) units. Hence, we used linear models such as principal component analysis (PCA) for further analysis as recommended by ter Braak and Šmilauer (1998). Macroinvertebrate densities were log₁₀ (*x* + 1) transformed to stabilize variances and normalize the data sets and rare taxa were not excluded from the analysis (Cao *et al.*, 1998, 2001; Cao, 1999; Nijboer and Schmidt-Kloiber, 2004). These analyses were conducted by the computer program CANOCO (for Windows, version 4.5, Plant Research International, Wageningen, The Netherlands). The response of the PCA scores of axis I to the relative contribution of the point source and environmental conditions was measured as the difference between reach means of the first axis scores for each sampling date.

Similarities in taxa composition between the upstream reach and the downstream reach among sampling dates were measured using the Jaccard's index of similarity (Townsend *et al.*, 1987). We compared individual macroinvertebrate densities and biomasses between the two reaches for each sampling date using the Bray–Curtis similarity (Bailey *et al.*, 1998) included in the SIMPER analysis routine of the computer package PRIMER (for Windows, version 5.2.2, Plymouth Marine Labs).

RESULTS

Physical and chemical parameters

Discharge was higher in spring and autumn in the two reaches. During the sampling period, discharge was 1.4 times higher on average at the downstream reach than at the upstream reach (paired *t*-test, *p* = 0.014; Table 1).

Table 1. Mean \pm SE, minimum (min.) and maximum (max.) values of physical and chemical parameters measured in the two reaches over the sampling period in La Tordera stream ($n = 6$)

Variable	Upstream			Downstream			<i>t</i>	<i>p</i>
	mean \pm SE	min.	max.	mean \pm SE	min.	max.		
Discharge (L/s)	174.8 \pm 56.8	50.1	414.9	249.3 \pm 65.1	83.1	508.4	-3.72	*
Temperature [†] (°C)	12.9 \pm 1.5	8.0	20.0	13.6 \pm 1.9	7.5	22.8	-1.27	n.s.
DO [†] (mg/L)	10.0 \pm 1.9	7.9	12.1	8.9 \pm 0.5	6.2	11.2	3.79	*
Conductivity (μ S/cm)	152 \pm 16.0	92.0	205.2	262.4 \pm 28.8	173.8	327.7	-7.44	**
DOC (mg/L)	1.23 \pm 0.32	0.55	2.39	1.91 \pm 0.25	1.01	2.71	-2.95	*
NH ₄ ⁺ -N (mg N/L)	0.04 \pm 0.01	0.01	0.07	0.65 \pm 0.31	0.15	2.14	-2.83	*
NO ₃ ⁻ -N (mg N/L)	1.22 \pm 0.23	0.74	2.20	2.65 \pm 0.38	1.72	4.11	-3.87	*
SRP (mg P/L)	0.010 \pm 0.002	0.006	0.015	0.262 \pm 0.106	0.052	0.628	-2.49	ns

Significance from paired *t*-test ($df = 5$) comparing the considered variables ($\text{Log}_{10}(x + 1)$ transformed) between the two reaches is also shown. n.s., not significant ($p > 0.05$).

* $p \leq 0.05$; ** $p \leq 0.01$.

[†]Values of temperature and DO correspond to values registered over 24 h cycles performed for each reach and sampling date.

Water temperatures were slightly lower in winter and higher in summer at the downstream reach than at the upstream reach. The downstream reach also had a higher diel temperature range, especially during the summer, but mean values were not significantly different (paired *t*-test, $p = 0.260$). Mean DO concentration at the downstream reach was consistently lower (1.1 mg/L lower on average) than at the upstream reach (paired *t*-test, $p = 0.013$). Conductivity was moderate in the two reaches, but it was almost two times higher at the downstream reach than at the upstream reach (paired *t*-test, $p = 0.001$). Nutrient concentrations were higher at the downstream reach than at the upstream reach on all dates, but the effect differed among nutrients (Table 1). On average, NO₃⁻-N concentration increased twofold below the point source (paired *t*-test, $p = 0.012$), while NH₄⁺-N concentrations increased 20-fold (paired *t*-test, $p = 0.037$). Although mean SRP concentration was much higher at the downstream reach than at the upstream reach, differences were only marginally significant (paired *t*-test, $p = 0.055$). The effect of the point source on DOC concentration was less noticeable but statistically significant (paired *t*-test, $p = 0.032$).

Density and biomass of benthic macroinvertebrates

We collected over 150 000 benthic macroinvertebrates distributed in 90 taxonomic groups in the two reaches over the six sampling dates. Macroinvertebrate density was significantly higher at the downstream reach than at the upstream reach on most sampling dates (HSD, $p < 0.026$) except in April and June 2002, when it was similar (HSD, $p = 0.370$ and $p = 0.957$, respectively; Figure 3a). Macroinvertebrate biomass was very similar in the two reaches over the sampling period (ANOVA HSD, $p > 0.130$; Figure 3b). However, mean biomass was much higher at the downstream reach than at the upstream reach in November 2001 and September 2002. On most dates, the downstream reach presented a higher ratio of density to biomass indicating predominance of smaller body size of benthic macroinvertebrates relative to the upstream reach.

The response of macroinvertebrate density and biomass to the point source was negatively correlated with the peak discharge of the previous spate ($R_S = -0.812$, $p = 0.050$ for the two descriptors; Table 2). The difference in macroinvertebrate biomass between the upstream and the downstream reaches was also negatively correlated with the relative contribution of DIN of the point source ($R_S = -0.829$, $p = 0.042$).

Diversity of benthic macroinvertebrates

Taxa richness was between 8 and 18 units higher at the upstream reach than at the downstream reach (paired *t*-test, $p = 0.001$; Figure 4a). In the two reaches, taxa richness was higher in winter and lower in spring. The rarefaction coefficient was higher at the upstream reach than at the downstream reach on all sampling dates (paired *t*-test, $p = 0.012$), but in June 2002, when it was similar in the two reaches (Figure 4b). Shannon diversity was much

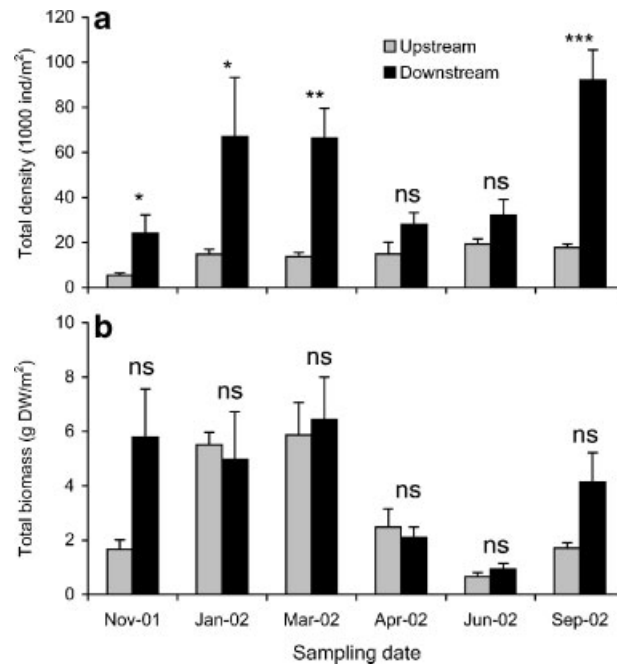


Figure 3. Mean \pm SE values of macroinvertebrate density (a) and biomass (b) in the two reaches of La Tordera stream over the sampling period. Asterisks indicate significant differences in density or biomass of macroinvertebrates between the two reaches (Tukey's HSD, n.s. = not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

higher at the upstream reach than at the downstream reach on three of the six sampling dates, but values were very similar between the two reaches in November 2001, April and June 2002 and were not statistically different between the two reaches (paired t -test, $p = 0.075$; Figure 4c). Similarly, the shapes of the Pielou's evenness curves of the two reaches through the sampling period followed the same pattern as Shannon diversity and differences between the two reaches were not statistically significant (paired t -test, $p = 0.162$; Figure 4d). At the upstream reach, Shannon diversity was strongly correlated with Pielou's evenness ($r = 0.992$, $p < 0.0005$), whereas the weight of taxa richness was not significant ($r = 0.384$, $p = 0.452$). In contrast, at the downstream reach the weight of the two components on Shannon diversity, evenness and taxa richness, were similar ($r = 0.998$, $p < 0.0005$ and $r = 0.832$, $p = 0.040$, respectively). EPT richness was between 6 and 10 units higher at the upstream than at the

Table 2. Two-tailed Spearman rank correlations between the difference of each community descriptor and centroids by date and reach of the sample PCA scores of axis I between the two reaches and the relative contribution of the WWTP effluent to the downstream reach in terms of discharge (rel. Q), dissolved inorganic nitrogen (rel. DIN) soluble reactive phosphorus (rel. SRP) and environmental conditions as mean water temperature (T) and peak discharge of the previous spate event (Q max)

	rel. Q	rel. DIN	rel. SRP	T	Q max
Δ Density	0.086	-0.371	-0.257	-0.257	-0.812*
Δ Biomass	0.314	-0.829*	-0.657	-0.429	-0.812*
Δ Taxa richness	0.463	0.062	-0.741	-0.926**	-0.329
Δ EPT richness	0.986***	-0.232	-0.203	-0.551	-0.618
Δ Shannon diversity	-0.257	0.314	-0.086	-0.257	-0.203
Δ Pielou's evenness	-0.200	0.486	0.029	-0.143	-0.145
Δ PCA scores (axis I)	0.371	-0.086	-0.714	-0.886*	-0.551

$n = 6$.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

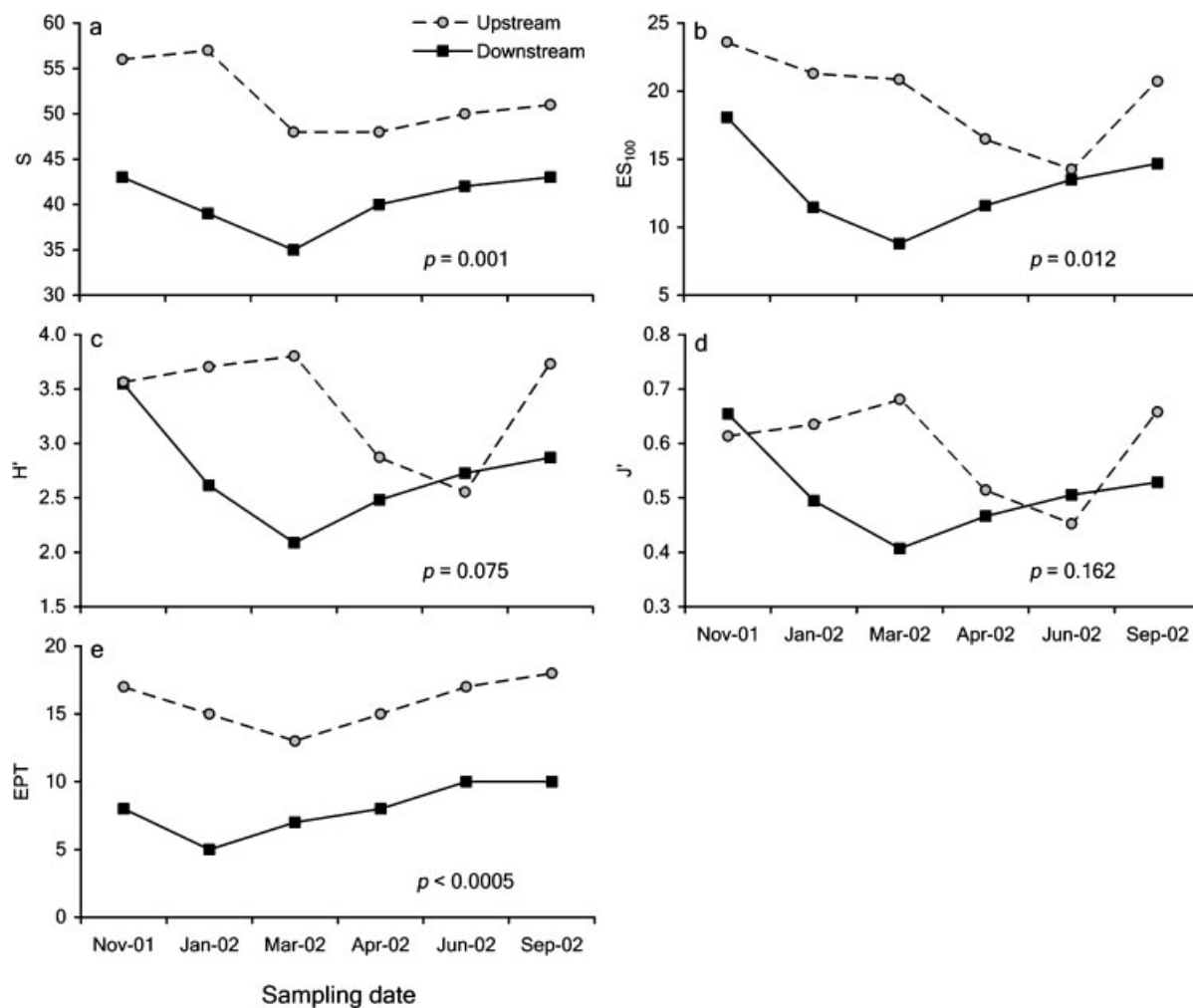


Figure 4. Taxa richness (S), rarefaction coefficient (ES_{100}), Shannon diversity (H'), Pielou's evenness (J') Ephemeroptera, Plecoptera and Trichoptera richness (EPT) in the two reaches of La Tordera stream over the sampling period. Results from paired t -tests are also shown

downstream reach (paired t -test, $p < 0.0005$; Figure 4e). The percentage of EPT taxa responsible for differences in taxa richness between the two reaches ranged from 46% in March 2002 to 100% in September 2002.

The response of taxa richness was negatively correlated with mean water temperature ($R_S = -0.926$, $p = 0.008$), whereas the response of EPT richness was higher with decreasing stream dilution of the point source ($R_S = 0.986$, $p < 0.0005$; Table 2). On the other hand, our results did not show any significant relationship between the response of Shannon diversity or Pielou's evenness with the relative contribution of the point source, water temperature or peak discharge of the previous spate event.

Ordination analyses

The PCA performed for 72 samples and 104 taxa explained 40.5% of the variance in the first two axes (axis I = 26.8%; axis II = 13.7%). Axis I was best explained by a positive relationship with *Dina lineata*, *Helobdella stagnalis*, Tubificidae, Nematoda, Nematomorpha, Tanytarsini and *Pisidium casertanum*, and by a negative relationship with *Serratella ignita*, *Ecdyonurus angelieri* and *Leuctra geniculata* (Figure 5a). Axis II was positively

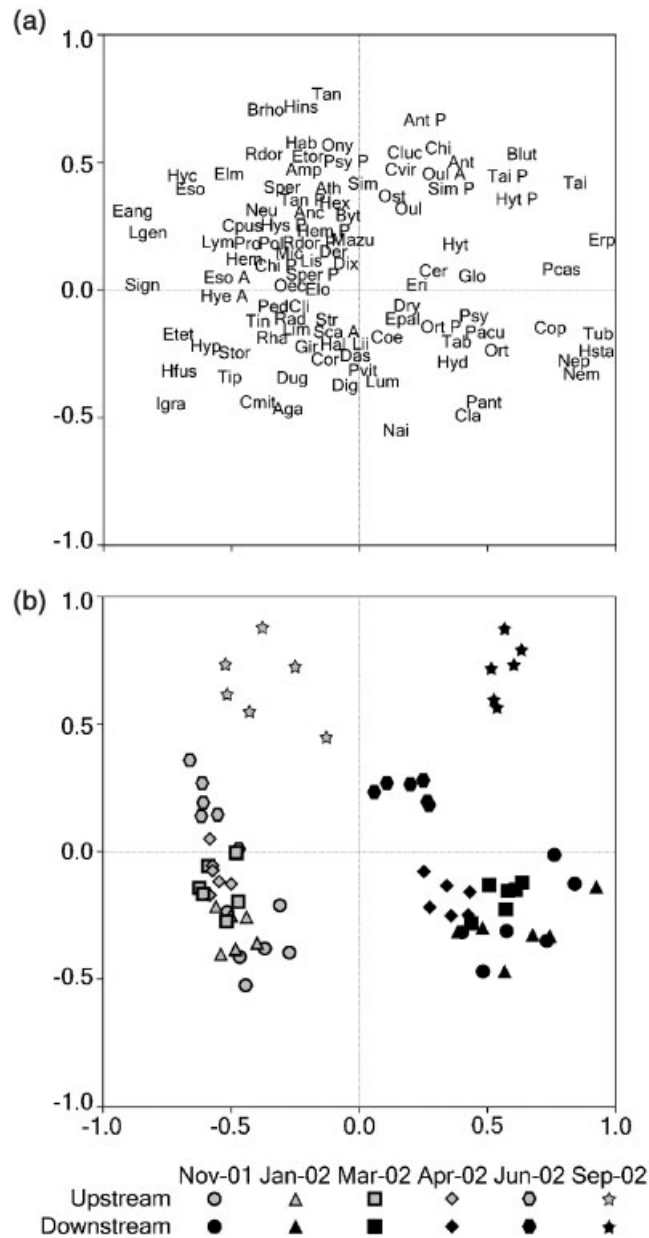


Figure 5. Principal component analysis (PCA) ordination of taxa composition (a) and macroinvertebrate samples (b) of the two reaches of La Tordera stream over the sampling period. Only taxa with a weight of 20% or higher are plotted. Aga = *Agabus* sp., Amp = *Amphinemura* sp., Ant = Anthomyiidae, Bfus = *Baetis fuscatus*, Blut = *Baetis lutheri*, Brho = *Baetis rhodani*, Chi = Chironominae, Cla = Cladocera, Cluc = *Caenis luctuosa*, Cmit = *Capnioneura mitis*, Cop = Copepoda, Cvir = *Calopteryx virgo*, Dlin = *Dina lineata*, Eang = *Ecdyonurus angelieri*, Elm = *Elmis* sp., Eso = *Esolus* sp., Etet = *Eiseniella tetraedra*, Etor = *Epeorus torrentium*, Hab = *Habroleptoides* sp., Hfus = *Habrophlebia fusca*, Hins = *Hydropsyche instabilis*, Hsta = *Helobdella stagnalis*, Hyc = Hydracarina, Hyt = *Hydroptila* sp., Igra = *Isoperla grammatica*, Lgen = *Leuctra geniculata*, Lym = *Lymnaea* sp., Nai = Naididae, Nem = Nematoda, Nep = Nematomorpha, Ony = *Onychogomphus* sp., Ort = Orthocladinae, Oul = *Oulimnius* sp., Pant = *Potamopyrgus antipodarum*, Pcas = *Pisidium casertanum*, Rdor = *Rhyacophila dorsalis*, Sign = *Serratella ignita*, Sim = Simuliidae, Sper = *Sericostoma personatum*, Stor = *Siphonoperla torrentium*, Tai = Tanytarsini, Tan = Tanyptodinae, Tip = Tipulidae, Tub = Tubificidae. A = adult, P = pupae

related to *Baetis fuscatus*, Tanyptodinae, *Hydropsyche instabilis* and *B. rhodani*, and negatively related to Naididae, Cladocera, *Potamopyrgus antipodarum*, *Agabus* sp., *Capnioneura mitis* and *Isoperla grammatica*.

Distribution of sites along axis I of the PCA clearly separated the samples from the two study reaches whereas axis II discriminates cases according to the temporal pattern (Figure 5b). Samples of the upstream reach plotted to the left while those of the downstream reach plotted on the right. Winter samples remained at the bottom of the diagram and summer samples were located at the top of the diagram.

No significant correlations were found between the relative contribution of discharge, DIN or SRP by the point source input and the PCA site scores of axis I, whereas mean water temperature explained a significant part of the variability of this response ($R_S = -0.886$, $p = 0.019$; Table 2).

Taxonomic composition

The most abundant taxon from November 2001 to March 2002 at the upstream reach was the chironomid subfamily Orthocladiinae (Table 3). Water mites sharply increased in April 2002 and *Baetis rhodani* was the dominant taxon in the samples of June and September 2002. At the downstream reach, the most common taxa were chironomids (subfamily Orthocladiinae and tribe Tanytarsini), tubificids, nematodes and nematomorphs, but like in the upstream reach, *B. rhodani* became dominant in June and September 2002. Twenty-four taxa were only found at the upstream reach including the mayfly *Epeorus torrentium*; most stoneflies such as *C. mitis*, *Siphonoperla torrentium* or *I. grammatica*, and many caddisflies such as *Rhyacophila dorsalis*, *Mystacides azurea* or *Sericostoma personatum*. However, after the spate of April 2002, some taxa that were previously restricted to the upstream reach, such as *L. geniculata* or *E. angelieri*, appeared at the downstream reach. Only four invertebrates were found only at the downstream reach (*P. casertanum*, *Glossiphonia* sp., tribe Eriopterini (Limonidae) and *Eubria palustris*). The Jaccard's index of similarity in community composition revealed higher resemblances between the two reaches in summer samples (0.62 of similarity) than in the rest of the samples (from 0.44 to 0.51 of similarity). The Bray–Curtis index of similarity in individual macroinvertebrate densities was more than two times higher in June 2002 (64.3%) than in the rest of the samplings (between 15.5% and 26.9%). Similarly, the Bray–Curtis index of similarity in taxa biomass between the two study reaches was higher in June 2002 (43.3%) while quite low on the other sampling dates (between 6.6% and 27.4%).

DISCUSSION

Effects of the point source on the benthic macroinvertebrate community

In La Tordera stream, degradation of water quality was moderate compared to other studies performed in nearby streams (Martí *et al.*, 2004). However, the effects of the point source were sufficient to lead to noticeable consequences on the macroinvertebrate community. The conditions at the downstream reach favoured taxa with small body sizes, short life spans and high reproduction rates such as midges or oligochaetes. As a result, macroinvertebrate density was much higher at the downstream reach than at the upstream reach. Several studies maintained that human impact decrease macroinvertebrate density (Garie and McIntosh, 1986; Kerans and Karr, 1994; Prenda and Gallardo-Mayenco, 1996). In contrast, other studies found that density is not affected by urbanization (Jones and Clark, 1987; Roy *et al.*, 2003), or that it may even increase under certain nutrient enrichment (Wiederholm, 1984; Miltner and Rankin, 1998; Morais *et al.*, 2004). Such contradictory results may be explained by the subsidy-stress hypothesis proposed by Odum *et al.* (1979). According to this assumption, certain community descriptors follow a response curve to usable inputs rather than a linear trend. Thus, macroinvertebrate density will increase under moderate nutrient enrichment and decrease under high nutrient inputs. Therefore, linear trends might result from studies performed on narrow ranges of organic pollution or influenced by other factors such as toxic compounds (Paul and Meyer, 2001).

An increase in food availability may allow consumers to invest less energy in searching for food, thus increasing their feeding efficiency (Wallace and Webster, 1996). This assumption may explain the increase of macroinvertebrate biomass below several point and diffuse sources in a plains stream of north central Colorado (Shieh *et al.*, 2003). In our study, macroinvertebrate biomass did not differ between the two reaches in any sampling

Table 3. Mean \pm SE density, biomass and taxa richness of each taxonomic order at the upstream and the downstream reaches and percentage of difference (upstream-downstream/upstream \cdot 100) in La Tordera stream over the six sampling dates

Group	Upstream			Downstream			Difference		
	Density	Biomass	Richness	Density	Biomass	Richness	Density	Biomass	Richness
Ephemeroptera	4623.0 \pm 797.6	439.7 \pm 63.3	5.9 \pm 0.2	8453.9 \pm 2306.6	149.1 \pm 22.7	3.8 \pm 0.3	-82.9	66.1	36.3
Plecoptera	643.3 \pm 89.1	97.1 \pm 15.3	2.6 \pm 0.1	4.3 \pm 3.1	1.0 \pm 0.5	0.1 \pm 0.0	99.3	99.0	96.8
Odonata	4.0 \pm 2.0	0.4 \pm 0.2	0.2 \pm 0.1	7.1 \pm 3.7	3.4 \pm 2.1	0.2 \pm 0.1	-77.8	-848.4	0.0
Coleoptera	214.1 \pm 31.8	38.6 \pm 9.4	2.9 \pm 0.2	46.1 \pm 11.9	7.2 \pm 2.1	0.7 \pm 0.1	78.5	81.5	76.2
Trichoptera	138.4 \pm 43.4	119.8 \pm 37.9	2.0 \pm 0.2	238.5 \pm 71.4	32.1 \pm 9.4	0.8 \pm 0.1	-72.4	73.2	60.3
Diptera	4450.2 \pm 448.1	471.5 \pm 91.2	7.7 \pm 0.3	29 547.3 \pm 4742.0	988.4 \pm 244.0	6.9 \pm 0.2	-564.0	-109.6	10.8
Mollusca	1177.9 \pm 199.9	933.8 \pm 178.9	3.6 \pm 0.2	1396.4 \pm 336.8	1393.3 \pm 370.2	3.6 \pm 0.2	-18.6	-49.2	0.0
Other taxa ¹	3066.1 \pm 729.3	873.2 \pm 184.7	8.0 \pm 0.3	11 944.1 \pm 2513.9	1480.6 \pm 185.6	10.6 \pm 0.3	-289.6	-69.6	-31.8

¹Includes minor taxonomic groups such as Oligochaeta, Hirudinea and Crustacea.

date. This result contradicts our initial hypothesis that macroinvertebrate biomass would also be enhanced under certain nutrient enrichment. Our results may indicate that macroinvertebrate biomass was limited by factors other than nutrient concentrations, but lack of related studies make it difficult to draw firm conclusions.

The changes induced by the point source into the stream ecosystem resulted in a decrease of taxa richness. The decline of EPT richness was in charge of a high proportion of the decrease of taxa richness at the downstream reach relative to the upstream reach. Although diversity indices were suggested as a useful metric to monitor water quality in the middle of the 20th century (Pinder and Farr, 1987; Lenat and Resh, 2001), they are rarely tested. A similar study in a southwestern Portuguese stream found that Shannon diversity and evenness were rather stable and were always lower in a reach affected by a WWTP input relative to a non-impaired reach (Morais *et al.*, 2004). In our study, Shannon diversity and evenness were highly variable and the differences between the two reaches were not consistent over the six sampling dates. Our results support the criticisms made by Lenat (1983) that diversity indices are not convenient for bioassessments because they can become highly reliant on factors other than organic pollution.

The point source input clearly affected taxa composition of the benthic community of La Tordera stream. At the downstream reach, several taxa were eliminated while others were enhanced. Stoneflies, mayflies and caddisflies were the most sensitive groups to the point source, in agreement with previous research (Lenat, 1983; Roy *et al.*, 2003). On the contrary, midges, oligochaetes, leeches and nematodes were favoured by nutrient enrichment, as has been reported in other studies (Hynes, 1978; Prenda and Gallardo-Mayenco, 1996). The conditions derived by the point source effluent at the downstream reach allowed the colonization of few taxa but were, at the same time, restrictive for a number of taxa. Indeed, taxa that appeared only at the downstream reach (e.g. *P. casertanum* and *Glossiphonia* sp.) were rather tolerant to pollution while the eliminated taxa (e.g. *E. torrentium*, *I. grammatica* and *S. personatum*) were considered sensitive to pollution (Tachet *et al.*, 2000).

Temporal variability

Our results show that community descriptors commonly used for bioassessment might show a high temporal variability that may not be directly related to organic pollution. At the downstream reach, macroinvertebrate density was higher in most, but not at all dates. Our results lend support to previous studies that flooding disturbance decrease macroinvertebrate density (e.g. Grimm and Fisher, 1989; Boulton *et al.*, 1992; Townsend *et al.*, 1998). Although detailed studies are required, our research suggests that the macroinvertebrate community of the upstream reach was more resistant and resilient to spates, in terms of macroinvertebrate density, than the community of the downstream reach. Such different response to flow disturbance led to similar macroinvertebrate density in the two reaches after the spates of April and May 2002. In this sense, the average low densities at the upstream reach may allow a faster recovery of the benthic community. The higher relative density of mayflies and stoneflies relative to dipterans and mollusks at the upstream reach may also have supplied a higher stability according to previous studies (Scarsbrook, 2002). We also detected higher similarities in terms of individual biomass after spates but, in contrast, macroinvertebrate biomass did not differ significantly between the two reaches for any sampling date. Flow disturbance occurring during the sampling period may have severely constrained macroinvertebrate biomass as reported by Grimm and Fisher (1989). However, the observed independence of macroinvertebrate biomass to nutrient concentrations was not simply a consequence of flow disturbance or macroinvertebrate biomass would have been higher at the downstream reach in January and March 2002. On the other hand, even though the biomass of the two reaches appeared to be equally affected by spates, at the downstream reach macroinvertebrate biomass increased faster than at the upstream reach. The faster recovery of macroinvertebrate biomass below the point source may result from an increase in productivity of nutrient enriched ecosystems as seen in previous studies (Shieh *et al.*, 2003). However, macroinvertebrate biomass of the two reaches seemed to be limited by the same asymptote.

Differences in taxa richness and EPT richness between the two reaches were relatively invariable. Consistent with the findings of Gasith and Resh (1999), at the upstream reach, taxa richness was higher in winter than in summer, and was not affected by the spates of April and May 2002. At the downstream reach, taxa richness was also relatively high in winter and decreased in March 2002, but increased again in the summer. Similarities in taxa composition between the two reaches were higher in the summer and the difference of taxa richness between the

reaches was significantly correlated with water temperature. These results contradict the findings of Gasith and Resh (1999) that the effect of pollution will be greater in the summer because stream dilution is lower in the Mediterranean climate. Such incongruence probably occurred because spates that took place in the spring of 2002 increased stream dilution improving water quality (Prenda and Gallardo-Mayenco, 1996), and because flooding may enhance drift from upstream reaches (Gasith and Resh, 1999). EPT richness was consistently higher at the upstream reach than at the downstream reach and the variability was relatively low in the two reaches. The difference in EPT richness between the two reaches seemed to be directly related to the effect of the point source on the stream. The high statistical power of taxa richness and EPT richness (Sandin and Johnson, 2000) along with the ease of calculation makes these two indicators good candidates for water quality assessments (Roy *et al.*, 2003).

The variation of Shannon diversity revealed a higher dependence on the evenness component than on taxa richness in the two reaches of La Tordera stream. The decrease of taxa richness and the increase of dominance by few taxa at the downstream reach resulted in a lower Shannon diversity than at the upstream reach on three dates. Morais *et al.* (2004) found that Shannon diversity and evenness increased at the impaired reach after flood disturbance. Our results show that these metrics did not only increase at the downstream reach but also decreased at the upstream reach after severe flow disturbances. Previous studies in a nearby river found that Shannon diversity was not related to pollution but with flow disturbance (Puig *et al.*, 1987). A study in a chalk stream in England (Pinder and Farr, 1987) attributed an increase of Shannon diversity below a WWTP input to a subsidy effect on oligotrophic waters. Although nutrient concentrations in our stream were comparable to that of the streams studied by Pinder and Farr (1987), we did not detect such a relationship. The observed independence of the Shannon diversity variability to the relative contribution of the point source suggests that it was driven by factors other than nutrient enrichment. In this sense, flow-related disturbance and population dynamics of certain taxa had important consequences on the structure of the benthic community in this stream. From July to October 2001, the two reaches were affected by a drought. The upstream reach completely dried and flowing water at the downstream reach came from the point source effluent. In November 2001, the increase of flow increased habitat suitability (Boulton, 2003) through expansion of the weighted area and translated to an unusually high evenness at the downstream reach. On the other hand, in April 2002, water mites suddenly increased at the upstream reach decreasing the evenness value in this reach. Such kind of alteration of the community structure due to the population dynamics of water mites has been reported in previous studies (Di Sabatino *et al.*, 2000). In addition, the spate of May 2002 considerably decreased the abundance of all macroinvertebrate taxa in the two reaches. This disturbance had harmful consequences on the structure of the macroinvertebrate communities, as has been described before in nearby streams (Argerich *et al.*, 2004). In June 2002, the communities of the two reaches were dominated by a large number of young *B. rhodani* nymphs. This translated to an important decay of evenness at the upstream reach, where the proportion of this mayfly relative to other taxa was much higher than at the downstream reach. The sudden dominance by *B. rhodani* may have simply been to population dynamics (Humpesch, 1979), or determined by the spate that occurred 1 month prior (Boulton *et al.*, 1992). Such erratic fluctuations in evenness led many authors to question the reliability of diversity indices in bioassessment (Lenat, 1983), and locate richness measures among the best indicators to detect water degradation (Sandin and Johnson, 2000; Ofenböck *et al.*, 2004). Overall, our findings lend support to the opinion of Collier and Quinn (2003) that regular sampling is required to obtain valuable assessments because population dynamics, hydraulic regime and natural disturbance may result in marked changes in the macroinvertebrate community and lead to misguided conclusions about the effects of human impact on stream ecosystems.

Few studies considered potential effects of flooding in streams affected by point sources. Although our study was not designed to assess the effects of flow disturbance, we detected higher similarities in total and individual densities and biomasses, taxa richness, EPT richness and community composition after the spates of April and May 2002. In addition, the response of macroinvertebrate density and biomass were negatively related to the magnitude of the preceding spate. These results suggest that intermediate frequencies and intensities of flooding (Townsend *et al.*, 1997) might act as a reset mechanism for macroinvertebrate communities (Lake, 2000). Flow disturbance is probably the most important organizing factor for stream ecosystems (Resh *et al.*, 1988) that could contribute in restoring stream benthos to a certain degree. However, although the use of flow disturbance can contribute to

ameliorate the ecological status of altered stream ecosystems, the potential effects of such disturbance on downstream ecosystems have to be considered as well.

CONCLUSIONS

The WWTP effluent of Sta. Maria de Palautordera and related outflows substantially increased discharge and nutrient concentrations in La Tordera stream. As a result, sensitive taxa declined while tolerant taxa increased. This translated in an increase of macroinvertebrate density but did not alter macroinvertebrate biomass. Calculation of different community metrics over regular samplings in combination with detailed analysis of hydraulic regimes, natural disturbances and population dynamics provide valuable information for stream bioassessments. There is now a substantial body of research on the effects of different human impacts on stream ecosystems but the multivariate nature of macroinvertebrate communities makes difficult to establish clear patterns for density, biomass and diversity indices. In contrast, the differences in taxa richness and EPT richness were consistent over the sampling period lending support to previous research that found these two indices as the most promising for biomonitoring. Shannon diversity and evenness plots supplied valuable information about the structure of macroinvertebrate communities but were highly dependent to factors other than nutrient enrichment.

The damaging effects of flooding and drying on macroinvertebrate communities have been well documented. However, our results revealed that flow disturbance could substantially contribute to amelioration of benthic community quality in human altered streams. The similarity between the two reaches, reference and altered, was higher after flooding. This happened because flooding scoured out the two reaches but also allowed certain taxa to colonize the downstream reach. We suggest that further research should focus on the study of the use of drying and flooding manipulations for future management plans understood as reset mechanisms for stream ecosystems.

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