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Pharmaceuticals and Personal Care Products in the Environment

BRIDGING LEVELS OF PHARMACEUTICALS IN RIVER WATER WITH BIOLOGICAL COMMUNITY STRUCTURE IN THE LLOBREGAT RIVER BASIN (NORTHEAST SPAIN)

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Abstract—A wide range of human pharmaceuticals are present at low concentrations in freshwater systems, particularly in sections of polluted river. These compounds show high biological activity, often associated with a high stability. These characteristics imply a potential impact of these substances on aquatic biota even when present at low environmental concentrations. Low flow conditions in Mediterranean rivers, most of which flow through densely populated areas and are subjected to intensive water use, increase the environmental risk of these emergent compounds. Here, we studied whether pharmaceuticals in river water affect the local benthic community structure (diatoms and invertebrates). For this purpose, we analyzed the occurrence of pharmaceuticals along the Llobregat River and examined the benthic community structure (diatoms and invertebrates) of this system. Some pharmaceutical products in the Llobregat River registered concentrations greater than those cited in the literature. Multivariate analyses revealed a potential causal association between the concentrations of some anti-inflammatories and β-blockers and the abundance and biomass of several benthic invertebrates (*Chironomus* spp. and *Tubifex tubifex*). Further interpretation in terms of cause-and-effect relationships is discussed; however, it must be always taken with caution because other pollutants also may have significant contributions. Combined with further community experiments in the laboratory, our approach could be a desirable way to proceed in future risk management decisions.

Keywords—Diatoms Invertebrates Llobregat River Multivariate analysis Pharmaceuticals

INTRODUCTION

Pharmaceuticals comprise an array of products, including a variety of chemical formulations and multiple biological targets. These drugs exert specific biological effects and are administered for human and veterinary health care. Although a variety of pharmaceutical compounds have been detected in the environment, their potential ecological significance remains unknown, and few studies have addressed their impact on nontarget species [1].

As a result of human population density and more intensive animal farming techniques, water catchments are highly susceptible to be at risk for potential contamination by various pharmaceutical products. A wide range of human pharmaceuticals, including analgesics, antibiotics, steroids, cardiovascular drugs, and various drugs used to treat mental illness, are present in effluents from sewage treatment plants that continuously enter freshwater systems [2–4].

Although concentrations of pharmaceuticals in the aquatic environment are generally in the nanogram-per-liter and low microgram-per-liter range, these compounds show high reactivity with biological systems; and are often highly stable. These characteristics may cause potential toxic effects to aquatic organisms even at low environmental concentrations [5]. Most studies in this field have addressed the effects of pharmaceuticals on aquatic vertebrates, namely, fish. Chronic toxicity data for algae and invertebrates have become more available in recent years, although they are usually obtained from aqueous-exposure experiments [6] rather than from field studies. Although pharmaceuticals are often moderately lipophilic [7], few studies have examined their potential impact on benthic communities and sediment organisms.

The rationale for the present study was to focus on algae and invertebrate fauna as representatives of aquatic benthic communities inhabiting the sediment interphase, with the aim to determine the potential effects of pharmaceuticals on the abundance and community structure of these organisms. These chemicals, transported by water, are adsorbed by particulate matter and accumulated in sediments. After adsorption, chemicals can be remobilized by resuspension or desorption, being a primary source of contamination for benthic organisms.

Analyses of chronic toxicity of pharmaceuticals on organisms are essential to obtain a realistic environmental risk assessment, because these substances were designed to exert distinct molecular modes of action. However, studies in this field usually provide data only for target species and work with concentrations far above those found in nature. Multispecies tests, even in standardized bioassays, allow realism in terms of ascribing biological endpoints to contaminant impact [8]. Data on the responses of natural communities in field conditions to pharmaceuticals may provide the information required to define the ecological relevance of these substances.

Given that flowing waters are exposed to multiple stressors, the effects of which can be cumulative and interact across spatial and temporal scales, defining causal relationships can be complex. To establish some relationships between environmen-

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tal stressors and biological response variables, it is necessary to integrate laboratory, field, and other experimental approaches such as mesocosms [9]. Field observations are essential for the detection of emerging consequences of stressors on communities and for the generation of hypotheses to identify potential cause-and-effect relationships.

The management of Mediterranean river basins requires consideration of their particular hydrology (low winter and summer discharges and periodical floods in spring and autumn) as well as continuing human pressure on resources and on the ecosystem. Characterized by low flows during normal conditions ($\sim$5 m^3/s) and extraordinary peak events (maximum recorded of 2,500 m^3/s in 1971) that periodically reset the system, the Llobregat River (northeast Spain) is a good example of a Mediterranean basin. The middle and lower sections of the river drain densely populated and industrialized (tannery, textile, pulp, and paper) areas. The major land use types in this area are: 38% urban and industrial activities and 13% farmlands. Furthermore, the Llobregat River provides drinking water to the large conurbation of Barcelona.

Several works have investigated the presence of various synthetic and natural estrogens and progestogens [10,11] and other pharmaceuticals [12,13] in scattered locations in the Llobregat River basin. The invertebrate community of this river has been used as indicator of its ecological quality since the early 1980s [14,15]. Diatom data from this river also have been available since the 1980s [16–18], and a surveillance program including macroinvertebrates and diatoms is currently implemented by the Catalan Water Authority.

Within this framework, here we aimed to analyze the occurrence of pharmaceuticals in the water in the Llobregat River basin and to find potential relationships between the presence of pharmaceuticals and the structural composition (changes in abundance and biomass) of the biological community (benthic algae and invertebrates) in this river. A total of 29 pharmaceuticals, including analgesics, anti-inflammatories, lipid regulators, antibiotics, psychiatric drugs, antihistamines, and β-blockers, were analyzed in river water. Other physical and chemical variables related to eutrophication and other environmental characteristics also were analyzed to delineate their influence on the natural community structure. Thus, to the best of our knowledge, the present study is the first to provide extensive data on the occurrence of a large number of pharmaceuticals in the aqueous phase of the Llobregat River basin and in a range of locations and time periods. On the basis of our findings, spatial and temporal variations in pharmaceutical load in the river can be deduced, together with the potential effects of these substances on biota.

MATERIALS AND METHODS

Study site

The Llobregat River (northeast Spain) is 156.5 km in length and covers a catchment area of approximately 4,948 km^2. The Llobregat River watershed is heavily populated (3,089,465 inhabitants in 1999). Together with its two main tributaries, the River Cardener and the River Anoia, the Llobregat River is a paradigm of overexploited Mediterranean rivers. The river has a mean annual discharge of 693,000,000 m^3, and nearly 30% is used for drinking water. The Llobregat River receives extensive urban and industrial wastewater discharges (137,000,000 m^3/year; 92% comes from the wastewater treatment plants) that cannot be diluted by its natural flow (0.68–6.5 m^3/s basal flow). Forty-eight percent of these point sources are located in the studied area. In addition, the middle part of the basin receives natural salt slurries from salt formations, which have caused an increase in water salinity downstream.

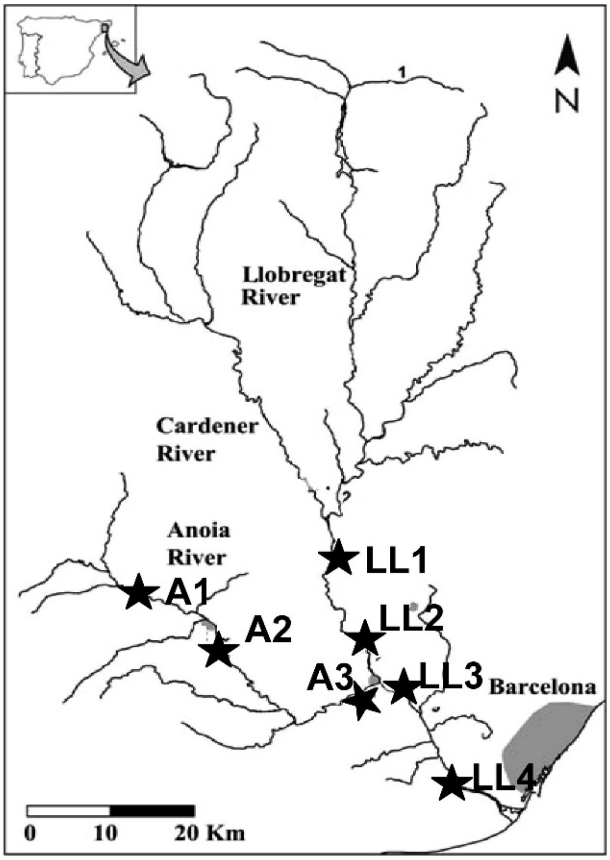

Fig. 1. Map of the sampling sites (Spain).

Four sampling sites were selected from the middle and lower parts of the Llobregat River main channel, and another three were selected in the River Anoia tributary (Fig. 1). These sites were part of a pollution gradient: Sites LL1 and A1 were the least polluted but received some industrial effluents and surface runoff from agricultural areas. Sites A3 and LL4 were located in the last section of the two rivers and were the most polluted sites. Site A2 was located in a highly polluted area receiving wastewaters from tannery, textile, and paper industries. Sites LL2 and LL3 were located in a densely inhabited area and received urban and industrial wastewater inputs.

Water samples for chemical (nutrients, cations, and anions) and pharmaceutical analysis were collected simultaneously with the biological samples from the sediments. Sampling was performed in early June 2005, November 2005, and late May 2006. These three periods covered two of the most relevant periods (spring and autumn) in the system in terms of its hydrology. Samples for grain size characterization were taken in the two first samplings. Ninety-eight percent of the sediment was gravel and sand in all sites. Sites A1, A2, LL1, and LL2 had a slightly higher proportion of gravel, and A3 had a higher proportion of fine sand respect to the other sites.

Water quality parameters

The pH, water temperature, conductivity, and oxygen concentration were measured in situ with appropriate probes. Water samples for nutrient analysis were collected in triplicate in each sampling period. The samples were filtered immediately (0.2-mm nylon membrane filters, Whatman) and frozen until analysis. Nitrate, sulfate, chloride, sodium, potassium, and calcium were determined by ion chromatography (761 Compact IC, Metrohm). Soluble reactive phosphate and ammonium were measured following standard procedures.

Analysis of pharmaceuticals

A total of 29 pharmaceuticals, belonging to the classes of analgesics and nonsteroidal anti-inflammatories, lipid regulators, psychiatric drugs, antihistamines, antiulcer agents, antibiotics, and β-blockers, were measured in water by means of off-line solid-phase extraction followed by liquid chromatography tandem mass spectrometry. Briefly, water samples (400 ml), previously passed through 0.45-µm nylon membrane filters, were preconcentrated on Oasis® hydrophilic–lipophilic balance (60 mg, Waters) cartridges, which were further rinsed with 5 ml of high-performance liquid chromatography-grade water, dried under vacuum for 15 to 20 min, and eluted with 2 × 4 ml of methanol. The extracts then were evaporated under a gentle nitrogen stream, reconstituted with 1 ml of methanol–water (25:75, v/v), and added to 10 µl of an internal standard mixture. Analysis of the extracts was performed by liquid chromatography–triple-quadrupole–tandem mass spectrometry, with an electrospray interface, operating in the selected reaction monitoring (SRM) mode. Two SRM transitions were monitored per compound. Nine of the 29 compounds were measured in negative ion mode, and the remaining 20 were measured in positive ion mode. This method and its validation (linearity, detection and quantification limits, repeatability, etc.) are described in detail in Gros et al. [19].

Diatom analysis

The sampling of biofilms for diatom analysis included those growing on large sediment particles (cobbles and gravel; epilithic biofilms) and sand (in the site LL4). Sand samples (2–5 cm in depth) were collected with a polyvinyl corer, and top subsamples were taken by an untapped syringe. Epilithic biofilm samples were collected by scrapping a known surface (1 cm^2) of gravel or cobbles with a knife. Samples were fixed with 4% formaldehyde for further identification and counting. One replicate from each sampling site was cleaned by means of acid oxidation. The cleaned frustules were mounted on resin (Naphrax, refractive index 1.74; Brunel Microscope) on permanent slides. The diatom community was observed and identified under a light microscope (Nikon Eclipse 600W) using phase-contrast and Nomarski differential interference contrast optics at a magnification of 1,000×. At least 400 valves were counted and identified to establish the relative abundance of the diatom species present in each sample.

Invertebrate analysis

Five sediment samples (10–15 cm in depth) were collected randomly with a polyvinyl sand corer in each sampling site. The animals retained when sieving the sediment through a 500-mm mesh (the benthic macrofauna) were fixed with 4% formaldehyde. The invertebrates were sorted, counted, and identified in the laboratory under a dissecting stereoscopic microscope. Identification was at family level for Oligochaeta and at the genus or species level for the remainder of the groups present (mainly Chironomidae and Ephemeroptera). Biomass was calculated as a dry weight from the length dimension using exponential equations [20]. Abundance and biomass were referred to the sediment surface area.

Data analysis

Diatom and invertebrate taxa accounting for more than 1% of total abundance in at least two of the samples were included in the analyses. Taxa abundances were square-root-transformed prior to analysis. A preliminary analysis based on the Bray–Curtis similarity index for abundance data was conducted to examine the extent to which macroinvertebrate and diatom assemblages varied among samples. This index calculates the similarity between two sites on the basis of species composition. A nonmetric multidimensional scaling (MDS) procedure was used to ordinate communities (and hence sites) on the basis of the similarity between each pair of samples estimated with the Bray–Curtis similarity index for abundances and Euclidean distances for invertebrate biomass data [8]. The MDS draws the sample distribution in a space delimited by a maximum of three axes. Short distances indicate high similarity in community composition between sites. The stress index measures the goodness of fit between the rank order of similarities and the rank order of distances [8]. Stress values higher than 0.3 indicate that the points are close to being arbitrarily placed in the two-dimensional ordination space.

Physicochemical variables, except pH, were transformed to reduce skewed distributions. Values of environmental and pharmaceutical variables were normalized by subtracting them from the mean and dividing this number by the standard deviation before their inclusion in the analyses. To avoid colinearity, the variables Ca^{2+} (correlated with Mg^{2+}, $r = 0.95$) and ranitidine concentration (correlated with sotalol, $r = 0.96$) were unselected. When the pharmaceutical concentrations were below method detection limits, a value equal to one-half of the method detection limit was assigned to these data in the statistical analyses.

The optimal match between the community patterns and the environmental variables associated with those samples was explored with the Bio-Env-Stepwise (BEST) procedure. This procedure searches for the highest rank correlation (Spearman correlation) between the species similarity matrix (calculated with abundances and biomass for invertebrates) and the environmental matrix (based on Euclidean distances). Spearman rank correlations were calculated by matching element to element [21]. The significance of the rank was assessed using the Monte Carlo permutation test (999 unrestricted permutations). These analyses were performed with the PRIMER® 6 statistical package (Plymouth Marine Laboratory).

In addition, invertebrate data were analyzed by detrended correspondence analysis to explore the species responses along an ordination axis. The maximum length of the gradient obtained with the detrended correspondence analysis was 2.44, indicating that linear methods were appropriate [22]. Consequently, we carried out a redundancy analysis, in which species data were constrained by environmental variables. This ordination assumes a linear combination of the species along the environmental gradients preserving the Euclidean distances. The redundancy analysis was performed with CANOCO® software, version 4.5 [22].

RESULTS AND DISCUSSION

Water quality parameters

Average values of the water quality parameters in the studied sites are shown in Table 1. The Llobregat and Anoia

Table 1. Physicochemical parameters and nutrients measured in the water samples collected at the selected sites. Average values and standard deviation (SD) are shown (n = 3). SRP = soluble reactive phosphate

	A1		A2		A3		LL1		LL2		LL3		LL4	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
pH	7.89	0.08	7.61	0.17	8.20	0.23	8.32	0.11	7.83	0.03	8.08	0.07	7.73	0.12
Temperature (°C)	14.67	1.12	23.07	3.49	21.87	4.88	20.33	4.65	20.77	3.36	21.30	3.41	24.47	2.76
Conductivity (µS/cm)	3,163.33	271.54	3,863.33	443.77	2,177.67	294.31	1,457.33	65.74	1,624.33	145.11	1,833.00	40.71	2,766.67	457.86
Oxygen (mg/L)	8.31	1.72	6.74	2.01	10.25	5.44	11.00	1.08	7.90	0.13	7.63	1.64	6.96	2.04
NO_3 (mg/L)	6.92	5.99	24.05	20.09	4.84	4.10	6.19	6.40	6.64	4.85	7.98	5.34	4.52	3.31
SO_4 (mg/L)	837.90	237.97	528.89	162.85	372.90	111.20	140.38	8.64	125.92	34.99	239.01	85.04	282.42	71.42
Cl (mg/L)	300.03	78.50	521.43	254.10	278.05	95.93	308.69	121.42	259.47	104.78	315.74	47.12	479.31	131.38
NO_2 (mg/L)	0.10	0.07	0.59	0.73	0.43	0.35	0.08	0.08	0.14	0.06	–	–	0.99	0.64
SRP (µg PO_4/L)	32.09	18.05	598.98	162.33	313.35	65.26	200.62	43.80	117.11	57.78	350.33	236.19	635.14	164.71
Na (mg/L)	195.38	30.02	349.87	202.38	209.85	76.08	131.26	13.45	146.53	43.10	168.80	42.68	284.65	40.95
K (mg/L)	12.12	9.23	23.65	13.33	28.42	24.97	45.27	25.11	46.42	29.36	23.06	19.39	72.47	50.23
Ca (mg/L)	207.64	21.70	119.34	37.85	113.72	24.87	68.24	4.09	59.98	7.07	71.84	10.41	77.37	6.67
Mg (mg/L)	79.16	19.46	45.68	13.83	43.45	13.13	24.09	6.17	23.15	7.52	29.25	9.66	27.24	13.30
NH_4 (mg/L)	0.33	0.32	0.95	0.06	0.47	0.41	0.24	0.34	0.67	0.54	2.31	1.32	0.65	0.18

River waters were characterized with high conductivity values and elevated ion concentrations. The upstream Anoia site had waters with high sulfate concentrations because of its gypsum bedrock. Salt (mainly KCl and NaCl) outcrops in the Llobregat River basin caused the high concentration of these ions and the corresponding high water conductivity.

The sites A2, LL3, and LL4 showed the highest nutrient concentrations and lowest dissolved oxygen concentrations. Water flow was low in the two spring seasons (2005, mean discharge in June of 4.79 m^3/s, and in 2006, 5.67 m^3/s, at the LL2 site), and nutrient loads and conductivity increased substantially from upstream to downstream reaches. In autumn 2005, the river discharge was higher (mean discharge in October, 8.71 m^3/s), and most of the concentrations reached their lowest values.

Pharmaceuticals in the Llobregat River

Twenty-one of the 29 compounds studied were present in at least one of the samples analyzed (Table 2). Twelve of these compounds presented maximum concentrations above 1 µg/L, and three (the analgesic diclofenac, the lipid regulator bezafibrate, and the antibiotic sulfamethoxazole) exceeded 10 µg/L. Mean concentrations higher than 1 µg/L were found for eight analytes. The highest mean concentrations were observed for ibuprofen (in sites A3 and LL4), diclofenac (A2 and LL4), clofibric acid (LL4), and ofloxacin (LL4), mainly as a result of punctual maximum concentrations in specific sites. Analgesics, anti-inflammatories, lipid regulators, and antibiotics were the families with the highest concentrations.

No significant seasonal variations in pharmaceutical occurrence were detected (analysis of variance, time as a factor, $p > 0.05$), but a clear spatial pattern was observed in sites A2, A3, and especially LL4, which registered the highest concentrations (analysis of variance, site as a factor and post-hoc comparisons with Tukey test, $p < 0.05$) (Fig. 2).

The pharmaceutical products observed in the Llobregat River closely matched those identified by the Spanish National Health System as those most consumed. These are mostly analgesics, antiulcer agents, antihistamines, antibiotics, and antidepressants. The concentrations of pharmaceuticals that we recorded in the Llobregat River were higher than those reported by Gros et al. [3] in the Ebro River, where concentrations ranged from 0.1 to 0.6 µg/L. In low flow periods in a Mediterranean river in France, Comoretto and Chiron [23] reported similar concentrations for carbamazepine and bezafibrate to those monitored in our present study. The concentrations of pharmaceuticals in the Llobregat River were in general in the range described by Fent et al. [2], except for those for ketoprofen, diclofenac, gemfibrozil, bezafibrate, and ranitidine, which were detected at higher mean concentrations in our present study.

Biological community structure in the Llobregat River

The ordination of the diatom assemblages (Fig. 3A) was derived from the two-dimensional MDS based on Bray–Curtis similarities calculated from square-root-transformed diatom abundances. Diatom ordination was highly similar between the least polluted sites (A1 and LL1), which showed the highest species richness. The community was dominated by the taxa *Navicula cryptocephala* in A1 and *Nitzschia inconspicua* in LL1. The community composition of site A2, one of the most polluted, clearly differed from those of the other sites.

The plot carried out with the data on invertebrate abundance (Fig. 3B) separated one group with the most polluted sites A2 and LL4. Most of the sites (except A1 and A3) in the autumn sampling formed a separate group related to the general low abundance found in these sites as a result of the higher discharge in that period. The remainder of the sites presented a more diverse community characterized by the presence of mayflies and several families of worms. The ordination carried out with the data on invertebrate biomass (Fig. 3C) showed sites A2 and LL4 to be separated from the others. These sites were characterized by the dominance of the nonbiting midge *Chironomus* spp. (mainly *bernensis* and *plumosus*), in terms of both abundance and biomass (Fig. 4A). Site A3 was characterized in the first spring sampling by the dominance of Oligochaeta (mainly *Tubifex tubifex*, Fig. 4B). This taxon also was present in the other sampling periods but with moderate values.

Communities of diatoms and invertebrates found in this study are characteristics of a perturbed fluvial system. There was a general decrease of species richness downstream and prevalent abundance of the most tolerant species to organic and chemical pollution. Salinity, high nutrient concentration, and low flow are considered to be responsible for biologically poor communities made up of tolerant taxa [15,17].

Table 2. Average, minimum, and maximum concentrations (μg/L) of pharmaceuticals in the water samples analyzed, and concentration at the 75th percentile ($n = 21$). MDL = method detection level

		Mean	Minimum	Maximum	75th percentile	MDL (ng/L)
Analgesics and anti-inflammatories	Ketoprofen	0.79	0.16	2.71	0.71	30
	Naproxen	0.53	0.02	2.06	0.65	7
	Ibuprofen	1.37	0.16	9.89	1.51	8
	Indomethacin	0.16	0.05	0.38	0.26	6
	Diclofenac	2.20	0.08	18.74	1.49	2
	Mefenamic acid	0.02	0.01	0.04	0.03	0.5
	Acetaminophen	0.42	0.06	2.42	0.45	17
	Propyphenazone	0.09	0.03	0.18	0.15	3
Lipid regulators and cholesterol-lowering statin drugs	Clofibric acid	2.28	0.01	7.91	3.29	1
	Gemfibrozil	1.42	0.04	7.78	1.42	1
	Bezafibrate	1.02	0.03	15.06	0.35	1
	Pravastatin	<MDL				47
	Mevastatin	<MDL				7
Psychiatric drugs	Carbamazepine	1.07	0.08	3.09	1.93	2
	Fluoxetine	<MDL				20
	Paroxetine	<MDL				8
Antiulcer agent	Lansoprazole	<MDL				5
Histamine H1 and H2 receptor antagonists	Loratadine	<MDL				2
	Famotidine	<MDL				5
	Ranitidine	0.11	0.01	0.57	0.09	2
Antibiotics	Erythromycin	0.03	0.01	0.07	0.06	4
	Azythromycin	<MDL				1
	Sulfamethoxazole	1.11	0.03	11.92	0.44	5
	Trimethoprim	0.14	0.02	0.47	0.21	1
	Ofloxacin	2.11	0.19	8.77	1.70	16
β-Blockers	Atenolol	0.22	0.05	0.67	0.32	9
	Sotalol	0.57	0.11	1.82	0.63	18
	Metoprolol	0.05	0.01	0.18	0.06	3
	Propranolol	0.03	0.01	0.06	0.06	2

Relationships between chemical and biological parameters

The results of the BEST procedure showed no significant correlation between the diatom community and the physicochemical and pharmaceutical variables. However, a significant rank correlation (0.492, $p = 0.001$) was observed between invertebrate abundance and the concentrations of indomethacin and propranolol, as well as with water temperature. Invertebrate biomass also showed a significant rank correlation (0.810, $p = 0.002$) with the concentrations of ibuprofen, atenolol, and propranolol. On the basis of the results from the BEST analysis, if the true driving abiotic variables are selected and two sites have very similar suites of values for these, then the assemblages also would be expected to be similar (and vice versa). This was the case in sites A3 and LL4, where the reduced invertebrate community, mostly made up of midge larvae (*Chironomus* spp.) and Oligochaeta showed higher abundances and biomass when the river carried higher concentrations of anti-inflammatories and β-blockers (Fig. 4C and D). Remarkably, temperature was the only selected environmental parameter related to invertebrate community abundance. This observation probably reflects the spatial arrangement of the polluted sites. Neither nutrients nor conductivity were selected as significant variables correlated with invertebrate community in these sites, regardless of their relevance as by-products of human activities in the Llobregat River and their established effects on the biological communities [15]. The finding that diatom distribution was not affected by the pharmaceutical products is also relevant; however, this observation may be attributed to the mode of action of these products, which does not directly affect the primary producers at the observed concentrations [2].

The redundancy analysis (RDA) determined that the concentrations of some anti-inflammatories and propranolol, as well as temperature, explained 71% of the taxonomic variance in invertebrate density (Fig. 5). The first RDA axis reflected the distribution of sites along the presence of indomethacin and propranolol but also on the increase of temperature. Ibuprofen was the variable most correlated with axis 2; temperature also was correlated with this axis (Table 3). Sites A2 and LL4 were associated with high concentrations of these pharmaceuticals, in contrast to the remaining sites. The position of the samples in the second axis was associated with the high concentration of ibuprofen in site A3, especially in the first sampling period. *Chironomus* spp. abundance was associated closely with the highest concentrations of propranolol and indomethacin and colder waters that were characteristic of site LL4 (Fig. 5B). Instead, the abundance of families of Oligochaeta (Naididae, Enchytraeidae, and Tubificidae) was found to be related to higher ibuprofen concentrations (site A3). The remaining taxa were related to low concentrations of pharmaceuticals (Fig. 5B).

The RDA analysis for diatoms showed a slight correlation of the environmental variables with the axis, and no significance of the Monte Carlo test was found.

The results of both multivariate analyses for invertebrate assemblage were similar; sites with higher concentrations of anti-inflammatories and β-blockers and higher temperatures were characterized by a greater abundance and biomass of *Chironomus* spp. and *Tubifex tubifex*. Several studies on the effects of chronic exposure indicate that ibuprofen has very little impact on aquatic environments [24–26]. However, an increase in the somatic growth of *Daphnia magna* population when exposed to ibuprofen (20 mg/L) and a reduction in reproduction have been described [27]. These authors suggested that the negative impact on reproduction would release energy to be invested in growth. Preliminary results (J.C. López-Doval, unpublished data) of a chronic laboratory test

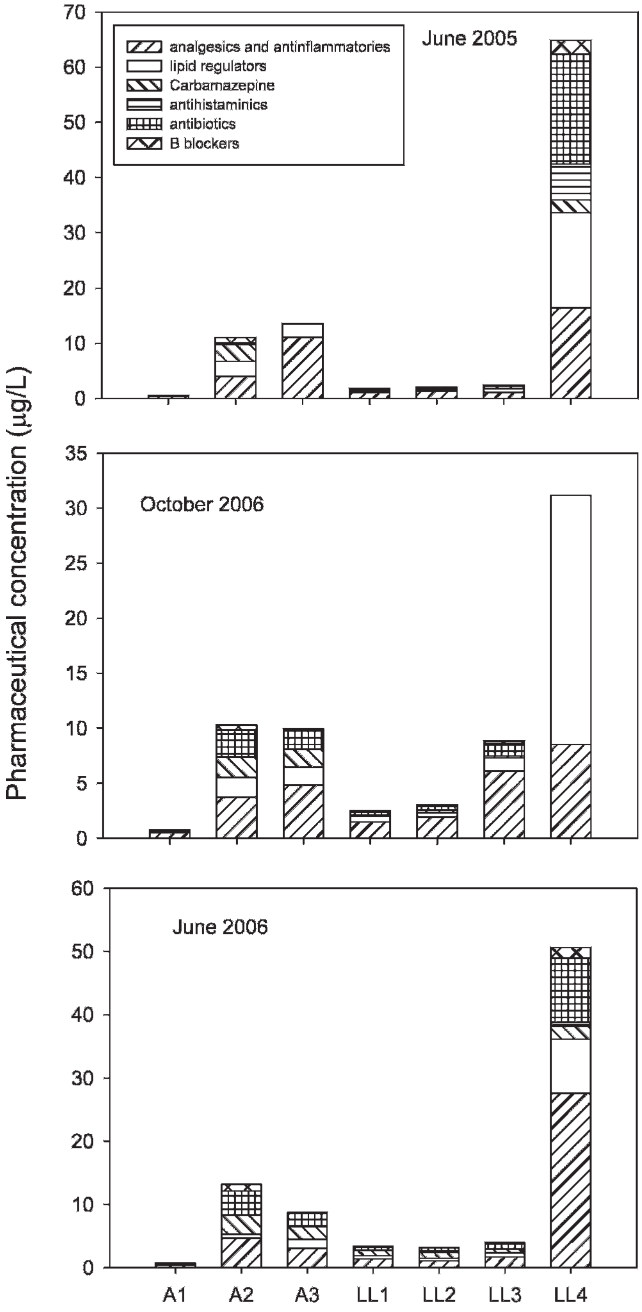

Fig. 2. Concentrations (μg/L) of the families of pharmaceuticals in the sites studied for the three sampling campaigns.

with *Chironomus riparius* exposed to sediments spiked with indomethacin (120 μg/g sediment) show an increase of nearly 60% of individual growth with respect to the control treatment.

Other authors listed numerous effects of anti-inflammatories in freshwater organisms; for example, Schwaiger et al. [28] and Triebskorn et al. [29] detected bioaccumulation and hystopathological alterations in kidney and gills in rainbow trout and common carp exposed to diclofenac (lowest-observed-effect concentrations, 1–5 mg/L) at a concentration range found in our present study. Pharmaceuticals in the environment may affect the same pathways in animals with similar target organs, tissues, cells, or biomolecules. Knowledge about these targets exists primarily for fish, but less is known in invertebrates or other phyla [2].

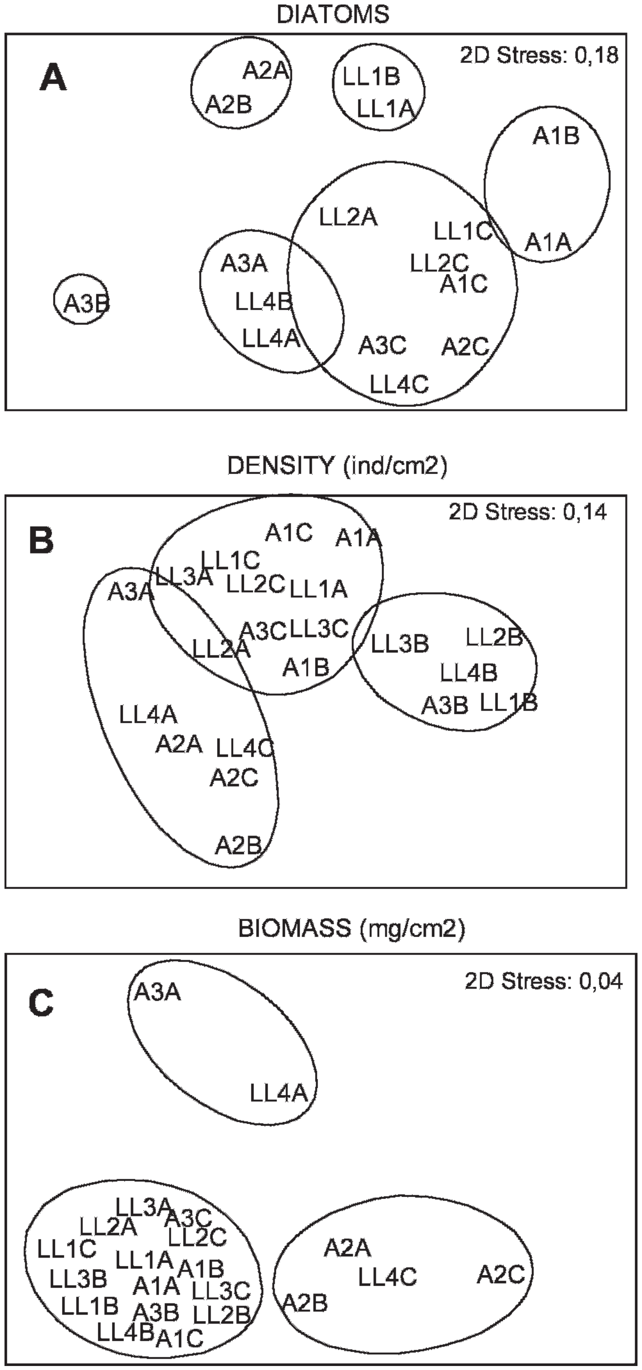

Fig. 3. (**A**) Nonmetric multidimensional scaling (MDS) ordination of the sites using Bray–Curtis similarities on root-transformed diatom species relative abundances. Lines delimit the groups formed by a cluster analysis (group average linked) at 40% of similarity. (**B**) The MDS site ordination for invertebrate abundance (individual/cm^2) using the same similarity index. (**C**) MDS site ordination using Euclidean distances for invertebrate biomass (mg/cm^2). Lines delimit the groups formed by cluster analysis at 1.1 distance index. Site codes: (**A**) spring 2005; (**B**) autumn 2005; (**C**) spring 2006.

The toxicity of the β-blockers is difficult to ascertain in invertebrates, because most studies have analyzed the effects only in *D. magna* [30]. Propranolol shows the highest acute toxicity and highest log K_{OW} among β-blockers. Stanley et al. [31] found a 48-h propranolol median effective concentration value for *D. magna* of 1.67 mg/L. These experimental concentrations were extremely higher than those that we registered in the Llobregat River water. β-Blockers may have

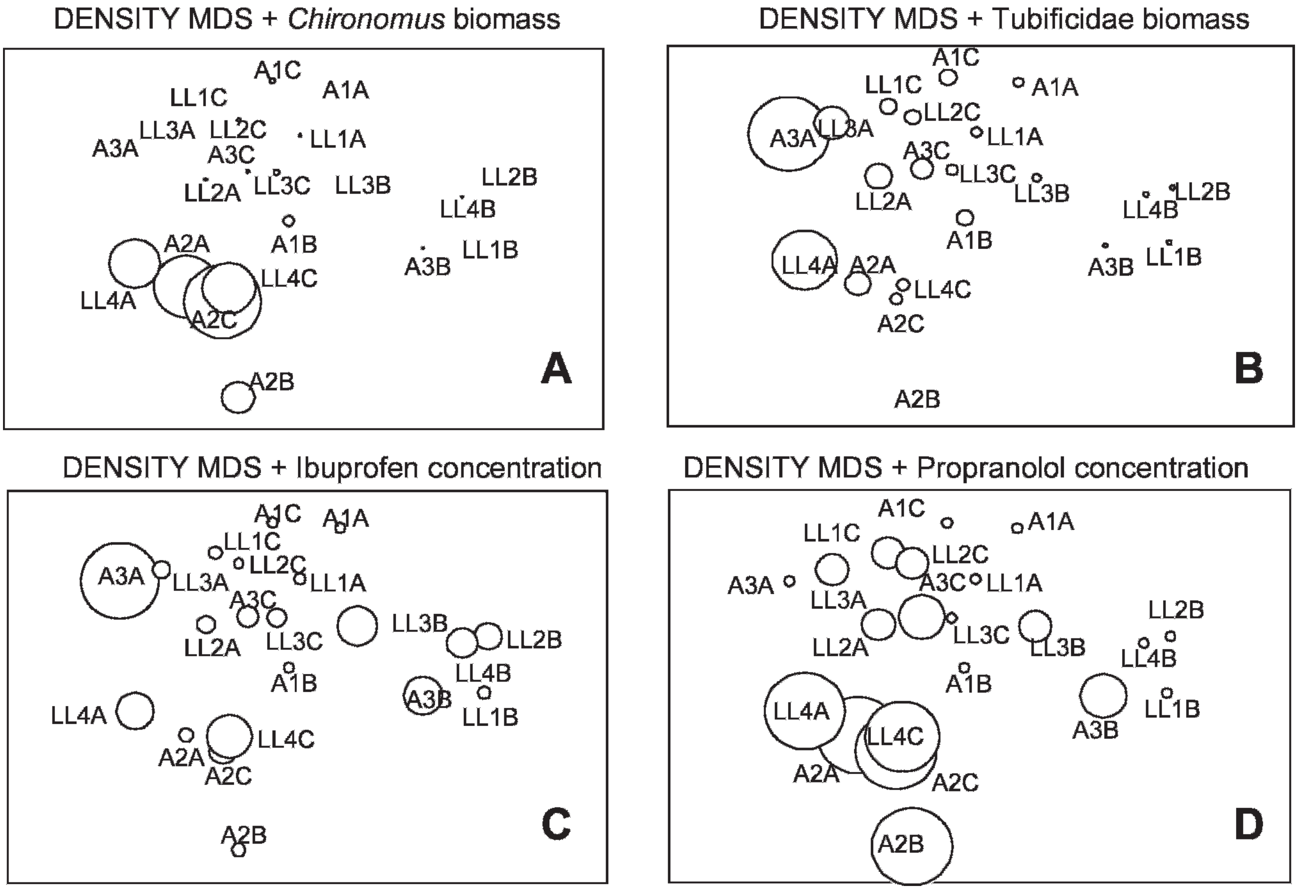

Fig. 4. (**A** and **B**) Invertebrate abundance multidimensional scaling (MDS) plot from Figure 3, with values of *Chironomus* spp. and Tubificidae biomass (mg/m^2) at distinct sites, superimposed as circles of different sizes on the basis and proportional to biomass values. (**C** and **D**) The same MDS plot on the basis and proportional to values of ibuprofen and propranolol concentrations (μg/L) in water samples.

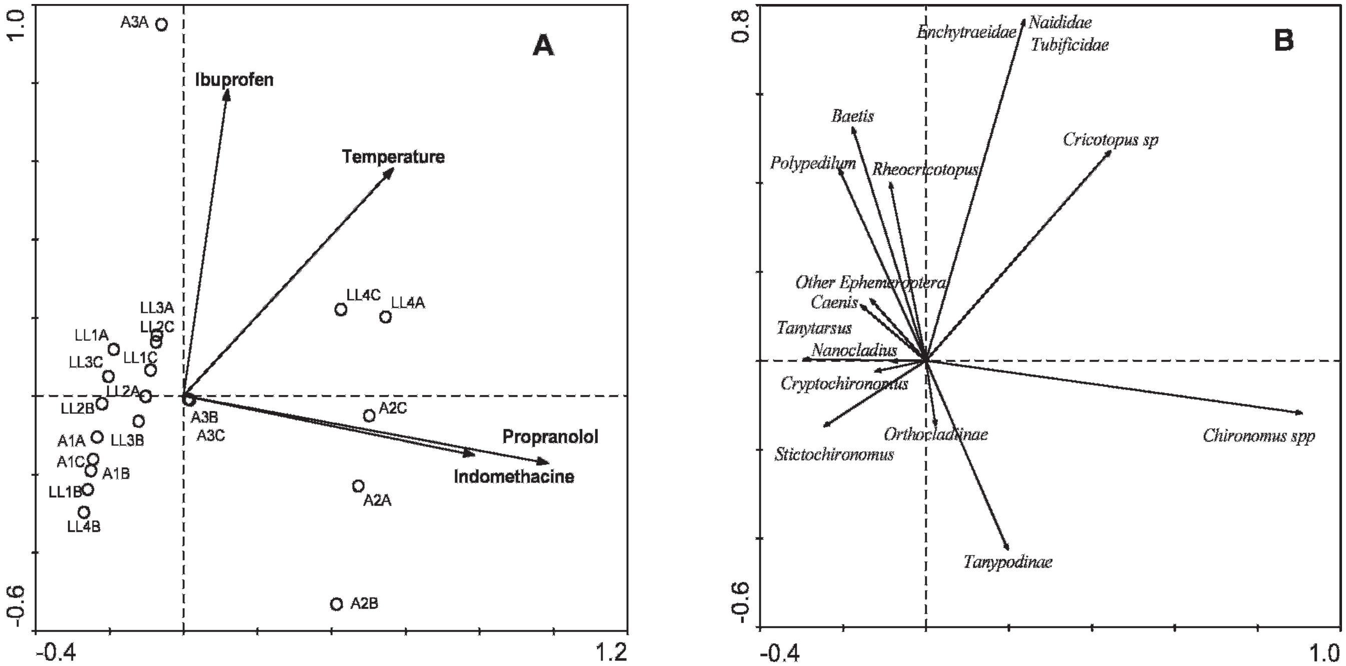

Fig. 5. (**A**) Redundancy analysis (RDA) ordination plot for sites and environmental relationships based on the abundance of invertebrate taxa. The environmental variables more significantly correlated with the RDA axes are represented in the plot by arrows, which point in the direction of maximum change in the value of the associated variable. (**B**) Ordination of invertebrate species on the first two environmental axes of the RDA.

Table 3. Correlation between axes and environmental variables after redundancy analysis of invertebrate species abundance data from Llobregat River

	Axis 1	Axis 2
Propranolol	0.90	−0.13
Ibuprofen	0.11	0.63
Indomethacin	0.72	−0.12
Temperature	0.52	0.47
Species environment	0.92	0.81
Eigenvalues	0.44	0.22

several effects on fish, such as cardiovascular dysfunction and impairment of fitness [32] or reproduction [33]. Propranolol swells fish erythrocytes, thereby affecting oxygen uptake.

Some psychiatric drugs, such as carbamazepine, show low acute toxicity in aquatic organisms, particularly invertebrates [34]. However, in response to chronic sediment exposure, the midge *C. riparius* shows a blockage of pupation and emergence (10% effective concentration values 70–210 mg/kg dry wt). Although information on the concentrations of pharmaceuticals in sediment is limited, it is feasible that these compounds accumulate in this compartment [35], thereby posing a risk for the survival of populations of benthic organisms.

Some of the mechanisms of action of pharmaceuticals described in the literature could explain the association between these substances and the invertebrates found in the Llobregat River. However, the correlational findings also could be the result of cumulative or synergistic effects caused by several stressors that co-occur in the system. Pharmaceuticals do not appear as isolated compounds in river water but as a mixture, and data on the responses of aquatic organisms to a mixture of pharmaceuticals are very limited. Escher et al. [36] reported higher toxicity of a pharmaceutical mixture than separated products. Flaherty et al. [37] indicated unpredictable and complex effects of a pharmaceutical mixture on *Daphnia* survival, growth, and reproduction. Moreover, other indirect effects can be influential factors, such as habitat and food availability, species competition, or predator–prey interactions. Although habitat characterization (sediment grain size) showed slight differences in the studied sites, little is known about the other parameters. Therefore, our results should be taken as indicative and require further experimental testing in controlled conditions such as mesocosms [38–40].

CONCLUSION

Several pharmaceutical products in the Llobregat River were found at concentrations higher than those cited in the literature. The low water flow of Mediterranean rivers increases the potential environmental risk of these emerging water contaminants. Thus, much of the aquatic biota will be exposed, throughout their lives, to complex mixtures of these compounds. Like other contaminants, the best way to reduce the ecological impact of pharmaceuticals in rivers may be to prevent input by improving sewage treatment procedures.

One of the major objectives for environmental scientists is to establish causal links between stressors and the quality of ecological systems. Procedures that identify causality allow corrective action measures for habitat recovery and control. Identifying the cause of biological impairment in freshwater systems is also an essential objective of the European Water Framework Directive. Our results reveal a potential causal relationship between the concentrations of a number of pharmaceutical products and the abundance and biomass of several key benthic invertebrates. Although our assessment has been based on field data, it provides evidence on how pharmaceuticals disrupt biological communities. Although strict guidelines for developing cause-and-effect relationships are not well established, some criteria support causality in environmental impact studies [40]. Experiments that include evidence from multiple field and laboratory studies are stronger than those based alone on only one kind of data. Knowledge of field patterns will help us to focus subsequent monitoring efforts and to identify key taxa for use as ecological indicators. This approach might be useful to find spatial and temporal correlations of stressors and effects along gradients, although it should be combined with community experiments in the laboratory to examine hypotheses generated from field studies. This could be a desirable way to proceed in future risk management decisions for these emerging water contaminants, taking into account both the scarcity of experimental studies with durations longer than a few weeks and the few works that used multiple species in standardized bioassays.

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