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A mesocosm approach for detecting stream invertebrate community responses to treated wastewater effluent

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A B S T R A C T

The discharge of wastewater from sewage treatment plants is one of the most common forms of pollution to river ecosystems, yet the effects on aquatic invertebrate assemblages have not been investigated in a controlled experimental setting. Here, we use a mesocosm approach to evaluate community responses to exposure to different concentrations of treated wastewater effluents over a two week period. Multivariate analysis using Principal Response Curves indicated a clear, dose-effect response to the treatments, with significant changes in macroinvertebrate assemblages after one week when exposed to 30% effluent, and after two weeks in the 15% and 30% effluent treatments. Treatments were associated with an increase in nutrient concentrations (ammonium, sulfate, and phosphate) and reduction of dissolved oxygen. These findings indicate that exposure to wastewater effluent cause significant changes in abundance and composition of macroinvertebrate taxa and that effluent concentration as low as 5% can have detectable ecological effects.

1. Introduction

Wastewater treatment plants (WWTPs) have been critical in reducing the impacts of raw sewage effluent on rivers and streams, yet the growth and concentration of human populations have led to a steady increase in effluent discharge volumes to the environment (Carey and Migliaccio, 2009). Effluent from WWTPs are highly enriched in nutrients and are estimated to account for over 50% of nitrogen and phosphorus loads to freshwater ecosystems (Martí et al., 2010). Furthermore, wastewater effluents often contain toxic contaminants, including pharmaceuticals, detergents, and flame retardants that are not effectively treated by WWTPs (Paxéus, 1996; Garric et al., 1996; Meyer and Bester, 2004; Kümmerer, 2009). Elevated levels of nutrients and pollutants introduced by wastewater discharges have been shown to promote eutrophication (Smith et al., 1999), alter fish and invertebrate community composition (Kosmala et al., 1999; Gafny et al., 2000; Brown et al., 2011), modify nutrient-processing dynamics (Ruggiero et al., 2006; Carey and Migliaccio, 2009), and alter primary production (Masseret et al., 1998). The degradation of rivers from sewage discharges also threatens key environmental services including water supplies for drinking and irrigation and recreational opportunities (Postel and Carpenter, 1997). Thus, the environmental impacts of wastewater effluent represent a critical and growing challenge to the sustainable management of water resources and conservation of freshwater biodiversity (Dudgeon et al., 2006; Postel, 2007).

The adverse effects of WWTP discharges on freshwater ecosystems and the services they provide are intensified under water scarce conditions, which are common to arid and semiarid climates such as the Mediterranean (Prat and Munné, 2000; Martí et al., 2010). Mediterranean regions are characterized by predictable summer droughts in which water is limited and flows in streams and rivers naturally become low or intermittent (Gasith and Resh, 1999). During the dry season, the capacity of recipient streams to dilute pollutants decreases, while waste water inputs remain constant or increase. This reduced-dilution effect is often exacerbated by water diversions for irrigation and consumptive uses, which can further limit natural flows (Gasith and Resh, 1999; Prat and Munné, 2000). Wastewater effluents have been reported to account for over 50%, and as high as 100%, of the discharge in Mediterranean streams during low-flow conditions (Martí et al., 2004; Canobbio et al., 2009; Carey and Migliaccio, 2009). As a consequence, WWTP discharges have an overwhelming influence on the hydrologic and nutrient regimes of streams and rivers in the Mediterranean region, especially during the summer (Kosmala et al., 1999; Prat and Munné, 2000).

The alterations in water quality and flow conditions resulting from wastewater discharges have been shown to have distinct effects on aquatic invertebrate communities. In general, exposure

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to wastewater effluents reduces species richness (Dyer and Wang, 2002) and causes a shift in community composition towards pollution-tolerant taxa such as Chironomidae and Oligochaeta (Cao et al., 1996; Ortíz and Puig, 2007). Most studies on WWTP impacts have involved sampling benthic macroinvertebrates over a disturbance gradient of wastewater discharge concentrations, such as upstream and downstream of an effluent outfall (Kosmala et al., 1999; Gücker et al., 2006; Ortíz and Puig, 2007; Pinto et al., 2010). However, such biomonitoring approaches may not be well-suited for detecting the effects of wastewater effluent when river ecosystems are influenced by other environmental and anthropogenic factors. For example, when macroinvertebrate communities are already impoverished by structural and chemical impacts, the apparent response to WWTP impacts may be weak or undetectable (Gücker et al., 2006). Strong seasonality, a distinctive characteristic of Mediterranean rivers, also complicates impact detection because seasonal variability in stream invertebrate assemblages can mask the effects of factors that may concurrently be affecting the community (Coimbra et al., 1996).

Stream mesocosms are artificial systems designed for controlled experiments and present an alternative to *in situ* sampling approaches for investigating the effects of potential stressors on aquatic communities (Odum, 1984; Petersen and Englund, 2005). Mesocosms have been extensively used in stream ecosystem research to improve understanding of the factors and processes that regulate benthic invertebrate communities (e.g., Lamberti and Steinman, 1993; Pearson and Connolly, 2000; Bond and Downes, 2003; Ledger et al., 2006). Stream mesocosm approaches have also been routinely employed to assess to the toxicity of potentially hazardous chemicals on aquatic macroinvertebrate taxa, such as pesticides (Richardson and Perrin, 1994; van den Brink et al., 1996; Colville et al., 2008), surfactants (Dorn et al., 1997; Belanger et al., 2004), metals (Richardson and Kiffney, 2000; Hickey and Golding, 2002; Brooks et al., 2004), and acid mine drainage (Perrin et al., 1992; Hruska and Dubé, 2004; Van Damme et al., 2008). Despite the successful application of mesocosms in stream ecosystem research, and ecotoxicology studies in particular, mesocosm experiments have not been used to evaluate the effects of WWTP effluents on aquatic invertebrates. Furthermore, previous research on the effects of treated wastewater on invertebrates has examined changes in taxa composition and abundance, but has not considered responses at the community level.

Here, we present a mesocosm experiment to evaluate the impacts of wastewater effluents on the benthic macroinvertebrate community of a Mediterranean-climate river in Catalonia, Spain. Effluent discharges from WWTP to rivers in Catalonia have been identified as a primary cause of aquatic ecosystem degradation and have put many rivers and streams at risk of failing to achieve target environmental quality conditions required by the European Water Framework Directive (WFD) (Prat and Rieradevall, 2006; Prat et al., in review). Therefore, quantifying how WWTP effluents affect invertebrate assemblages has important implications for the way rivers are managed in Spain and other Mediterranean-climate regions of Europe. The specific objectives of the study were to: (i) quantify the multivariate macroinvertebrate community response to varying dilution levels of WWTP effluent; (ii) measure taxa-specific responses to effluent impacts; (iii) identify the physical–chemical parameters that explain patterns in community responses to the experimental treatments; and (iv) quantify impacts in relation to biotic indices used for assessing the ecological status of rivers.

2. Methods

2.1. Study site

The stream mesocosm experiment was conducted at a wastewater treatment plant near the town of Balsareny (41°50′54″N, 1°52′49″E), located approximately 60 km north of Barcelona, Spain (Fig. 1). The Balsareny WWTP serves a population of 3410. Approximately 700 m^3 of sewage are treated daily through a combined process of primary treatment, in which heavy solids are settled and removed, and secondary treatment, in which suspended organic matter is consumed by microorganisms in a biological reactor. The WWTP is located on the banks of the Llobregat River, which it uses as both a water source and discharge point for treated effluent.

The Llobregat River and the ecological effects of pollution on its invertebrate communities have been studied for over 30 years (Prat et al., 1984; Prat and Ward, 1994). Long-term biomonitoring data for the Llobregat River indicate that the biological quality has remained in poor conditions for 25% of the 24 sampling sites (Prat and Rieradevall, 2006). While most of the sites located in upstream of Balsareny have good water quality conditions, the downstream sections of the river have been impacted by effluents from WWTPs and pollutant discharges from salt mines and other industrial activities (Prat and Rieradevall, 2006). A recent study was conducted in the spring of 2010 to evaluate the potential effects of the Balsareny WWTP on the benthic macroinvertebrate community (Caus and Prat, 2010). Macroinvertebrates were sampled in the Llobregat River approximately 1.3 km upstream (L1) and 300 m downstream (L2) of the WWTP outfall (Fig. 1). The L1 sampling point is located in a relatively pristine reach of the Llobregat River. Approximately 600 m downstream, there is a hydroelectric dam and diversion canal that conveys approximately 90 percent of the river flow for approximately 1 km until it is discharged back into the river. Thus, the river reach at the L2 sampling point is affected both by WWTP discharges and reductions in natural flows. Multi-habitat sampling of the benthic invertebrate community indicated that total density was higher at L2 but was dominated by pollution-tolerant taxa. However, most taxa had lower densities in the downstream site and total richness declined from 23 to 16 taxa. Overall, water quality and indices of ecological status were substantially lower downstream of the WWTP (Table 1).

2.2. Experimental design

To further examine the potential impacts of the wastewater discharges on the river macroinvertebrate community, a stream mesocosm was constructed at the

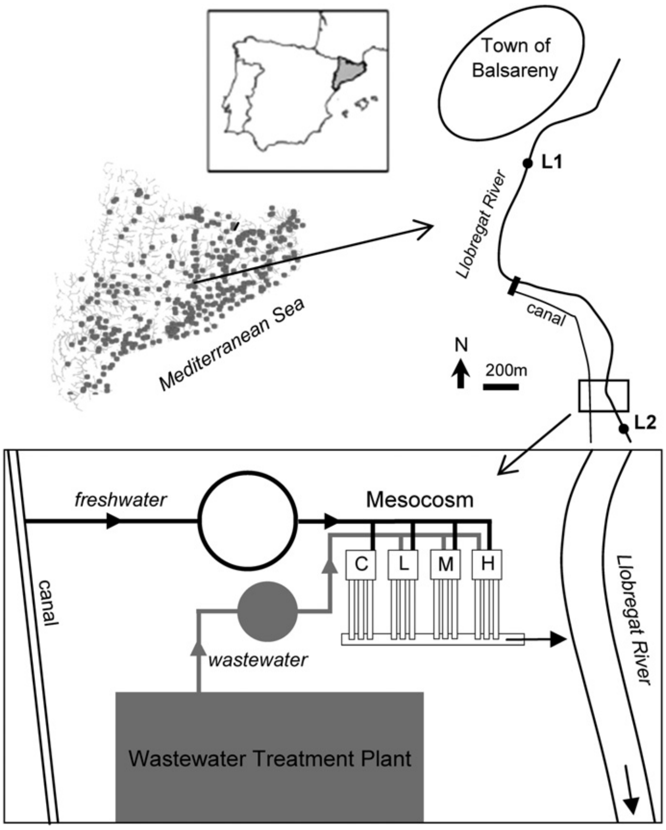

Fig. 1. Stream mesocosm at the Balsereny waste water treatment plant (WWTP) on the Llobregat River in Catalonia, Spain. River water was diverted from a diversion canal, mixed with wastewater effluent in four concentrations [0% (control; C), 5% (low; L), 15% (moderate; M), and 30% (high; H)] and continuously supplied to 12 artificial channels for 2 weeks. The site is located between biomonitoring sampling sites (L1 and L2). Municipal WWTPs in Catalonia are represented by gray dots on the inset map.

Table 1
Descriptive metrics of macroinvertebrate community upstream (L1) and downstream (L2) of the Balsareny WWTP, and corresponding ecological status.

Sampling location relative to WWTP	Discharge (l/s)	Diversity (*H*′)	Number taxa (*S*)	EPT taxa	IASPT	Log (Sel EPTCD + 1)	IMMi-t	IBMWP	Ecological status[a]
Upstream (L1)	3333	1.71	23	9	5.1	1.97	0.91	0.85	very good, good
Downstream (L2)	304	0.59	16	3	2.8	1.02	0.35	0.35	poor, poor

[a] Ecological status classification based on biotic index (IMMi-t, IBMWP) threshold values for Mediterranean rivers, developed by Munné and Prat (2009).

WWTP site in fall of 2009. The stream mesocosm consists of 12 artificial channels and is operated as a flow-through system, relying on water pumped from a diversion channel of the Llobregat River (Fig. 1). The pump provided a continuous supply of water to a 4000-L tank, fitted with an overflow pipe to maintain a constant water level and pressure head. Water was gravity-fed from the tank through polyethylene pipes to a series of four 96-L mixing tanks. Outlet pipes fitted with taps maintained flows from each mixing tank to the tops of three stream channels. Each channel consisted of a 2-m long, 12-cm wide by 8-cm deep polyvinyl chloride (PVC) drain trough. A second, 2000-L tank was filled with treated effluent from the WWTP and was connected to each of the mixing tanks through a secondary plumbing system. Taps on the inlet pipes regulated the proportions of river water and effluent entering each mixing tank, which created uniform concentrations in each tank before flowing into three artificial stream channels. Flows in each of the streams was maintained at a constant rate of 0.33 L/min, with corresponding hydraulic conditions similar to those present in natural riffles from the river of origin.

The experiment was carried out in the spring of 2010. On April 13, river cobbles (average surface area of 189 cm^2) were collected from riffle habitat in the Llobregat River and transported in individual mesh baskets to the stream mesocosm site. Nine cobbles were placed in each of the artificial stream channels and submerged by at least 10 mm of flowing water. Baskets with 1-mm vinyl mesh were placed at the outlet of each stream to capture invertebrate drift. The streams were fed by natural river water for one week to allow for colonization and the stabilization of invertebrate communities. Individual organisms collected in the drift baskets were relocated to the top of each channel every day during the settlement period. All twelve streams were used for the experiment and individual treatments were allocated to each of the four mixing tanks, yielding three replicates of each treatment. The experiment involved three treatments of wastewater effluent at dilution levels of 5%, 15%, and 30% concentration by volume, in addition to a control (river water only) group. The experimental waste water treatments were applied to the streams on April 15, 2010 and maintained for a two week period.

2.2.1. Macroinvertebrate and water quality sampling

Benthic macroinvertebrates were sampled from five cobbles collected from the river on April 13, 2010 and three cobbles in each of the mesocosm channels on April 22 and 30, representing approximately one and two weeks of treatment exposure, respectively. Each cobble was transferred to a 500-μm mesh bag and washed by hand for several minutes to remove the macroinvertebrates. The contents of the nets were emptied into bottles and preserved in formaldehyde. In the laboratory, macroinvertebrates were sorted and identified to the family or genus level. The raw taxa abundance data was scaled by the surface area of each cobble, measured with tin foil, to estimate the density of each taxa present (number of individuals per square meter).

Water quality conditions in each stream channel were measured during each macroinvertebrate sampling event. A YSI multimeter (63/10 FT) was used to measure *in situ* water temperature, conductivity, pH, and dissolved oxygen (DO). Water samples were also collected for each treatment group (from source water in the mixing tanks) and sent to a laboratory for chemical analysis to measure concentrations of ammonium, nitrate, nitrite, chlorine, sulfate, and soluble reactive phosphate.

2.2.2. Data analysis

The macroinvertebrate community data from the mesocosm experiment were analyzed using the Principal Response Curve (PRC) method (van den Brink and TerBraak, 1999), which is designed to evaluate the temporal responses of communities to controlled, experimental treatments. The PRC method is a form of redundancy analysis and generates series of curves that represent the extent and direction of differences between experimental treatments and controls. It is a useful method for visualizing the trajectories of community responses to treatments over time, and is commonly used in stream mesocosm studies (e.g., van den Brink et al., 1996; Colville et al., 2008; Duarte et al., 2008). The PRC analysis was performed in CANOCO version 4.5 (Ter Braak and Šmilauer, 2002) with log-transformed ($x + 1$) macroinvertebrate data, using average densities from the three cobbles sampled per channel, per sampling date.

The resulting PRC diagram displays the first principal component of each treatment effect over time, and can be interpreted as the deviation in the community response from the controls. In addition, species weights are calculated for each invertebrate taxa that indicate how closely the response of that taxa follows the overall community response defined by the PRC. Taxa with a high weight are inferred to exhibit changes in abundance that follow the pattern of the PRC, whereas taxa with species scores near zero either show no response to the treatment or a response that is unrelated to the PRC (van den Brink and TerBraak, 1999).

To determine the overall significance of the PRC in describing community trends, Monte Carlo permutations were performed with macroinvertebrate density data from each replicate channel. The significance of treatment effects at each sampling date was also assessed by Monte Carlo permutation tests, restricting the data to each sampling date. When the permutation tests indicated significant treatment effects, additional statisical tests were performed to evaluate differences between treatment groups and the control. Because of the limited number of replicates, permutation tests could not be performed for between-group comparisons and univariate methods were used. The multivariate data were reduced to a single variable by calculating the first component of the PCA for each sample. A one-way analysis of variance of the PCA scores, followed by Dunnett's post hoc tests, was then conducted to determine which treatment groups were significantly different from the controls.

The physicochemical data from water quality samples were also analyzed with PRC to assess the correlation between the physicochemical parameters (explanatory variables) and treatment effects. The data were square-root transformed and standardized for the PRC analysis. As with the macroinvertebrate data analysis, the PRC diagram describes the multivariate response of the water quality parameters to each treatment relative to the control. The species scores indicate which parameters had the greatest response to the treatment and identify which chemicals may be most important in controlling changes in the macroinvertebrate community.

In addition to the multivariate analysis, several univariate community metrics were calculated, including taxa richness (S), Shannon Diversity (H′), and number of Ephemeroptera, Plecotera, Trichoptera (EPT) taxa, using pooled samples from each treatment group. The diversity metrics were then used to calculate several indices of biological quality commonly used to assess the ecological status of rivers in Spain, including IBMWP and IMMi-t (Munné and Prat, 2009). Finally, the biotic indices for each treatment group were evaluated in relation to an ecological quality classification system based on the European Water Framework Directive (Munné and Prat, 2009).

3. Results

3.1. Macroinvertebrate community response to effluent treatments

A total of 17 taxa were identified among all treatment groups during the mesocosm experiment (Table 2). The relative abundance of taxa changed substantially in both treatment and control groups over time, suggesting the combined influence of natural colonization processes and experimental effects. Oligochaeta and Chironomidae were the most numerically-dominant taxa in all samples and had distinct treatment responses. After one week of exposure, the density of Oligochaeta increased to over 28,000 ind/m^2 in the 30% treatment group, indicating a strong positive response to the effluent treatment. However, by week two the abundance of Oligochaeta both in the 15% and 30% treatment groups declined dramatically, while the control and 5% treatment exhibited a slight increase in abundance. Chironomidae had relative similar densities in all groups after one week, but treatment effects became evident by week two. Between week one and two, there was an increase in Chironomidae densities that appeared to be mediated by effluent concentrations. The greatest increase in densities was observed in the control, followed by the 5% and 15% effluent treatment groups. In contrast, Chironomidae densities in the 30% treatment group slightly declined. Coleoptera and Ephemeroptera showed a strong concentration-dependent decrease in density and by week 2 were completely eradicated from the 30% treatment group. The density of Diptera taxa also decreased in response to the treatments, with

Table 2
Densities of invertebrate taxa (mean ± SD individuals/m^2) for each treatment group at 0, 1, and 2 weeks of exposure to wastewater effluent.

	Week 0	Week 1				Week 2			
	River control	Control	5% Effluent	15% Effluent	30% Effluent	Control	5% Effluent	15% Effluent	30% Effluent
Oligochaeta									
Oligochaeta spp.	5468 ± 2858	3664 ± 1924	5012 ± 1660	22,717 ± 4461	28,030 ± 7905	9967 ± 2519	10,348 ± 3895	11,703 ± 3894	2156 ± 1733
Mollusca									
Ancylidae	0 ± 0	0 ± 0	4 ± 7	5 ± 9	0 ± 0	27 ± 47	0 ± 0	0 ± 0	0 ± 0
Hydrobiidae	7 ± 16	0 ± 0	2 ± 3	4 ± 4	11 ± 14	2 ± 4	2 ± 3	10 ± 12	2 ± 3
Ephemeroptera									
Baetidae	266 ± 200	34 ± 48	21 ± 9	37 ± 12	14 ± 12	146 ± 54	56 ± 11	17 ± 11	0 ± 0
Caenidae	7 ± 15	4 ± 4	7 ± 8	4 ± 7	0 ± 0	8 ± 4	2 ± 3	4 ± 7	0 ± 0
Coleoptera									
Elmidae	94 ± 86	29 ± 5	5 ± 5	22 ± 24	36 ± 17	32 ± 13	55 ± 51	7 ± 9	0 ± 0
Heteroptera									
Aphelocheiridae	7 ± 16	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Trichoptera									
Brachycentridae	9 ± 20	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Hydropsychidae[a]	70 ± 68 (0 ± 0)	5 ± 10 (0 ± 0)	20 ± 7 (0 ± 0)	11 ± 5 (0 ± 0)	14 ± 8 (2 ± 3)	9 ± 8 (0 ± 0)	10 ± 3 (0 ± 0)	4 ± 7 (0 ± 0)	3 ± 6 (0 ± 0)
Hydroptilidae[a]	464 ± 187 (0 ± 0)	99 ± 45 (2 ± 3)	62 ± 62 (2 ± 4)	113 ± 143 (0 ± 0)	97 ± 4 (2 ± 3)	96 ± 55 (8 ± 9)	118 ± 49 (6 ± 7)	52 ± 51 (41 ± 40)	37 ± 38 (21 ± 23)
Philopotamidae	0 ± 0	0 ± 0	2 ± 4	0 ± 0	0 ± 0	4 ± 6	0 ± 0	0 ± 0	0 ± 0
Polycentropodidae	50 ± 93	2 ± 4	0 ± 0	5 ± 9	2 ± 3	2 ± 3	2 ± 3	0 ± 0	0 ± 0
Psychomyiidae	1114 ± 688	605 ± 290	633 ± 147	400 ± 121	40 ± 52	470 ± 244	157 ± 29	6 ± 10	0 ± 0
Rhyacophilidae[a]	143 ± 143 (0 ± 0)	14 ± 6 (4 ± 3)	6 ± 1 (13 ± 12)	13 ± 3 (17 ± 18)	16 ± 11 (33 ± 52)	13 ± 11 (0 ± 0)	2 ± 3 (10 ± 9)	2 ± 3 (4 ± 6)	2 ± 3 (2 ± 3)
Diptera									
Ceratopogonidae	0 ± 0	3 ± 5	7 ± 8	0 ± 0	6 ± 10	17 ± 29	10 ± 4	4 ± 3	4 ± 3
Chironomidae	12,320 ± 6101	6387 ± 1974	8429 ± 2791	6743 ± 570	4263 ± 1711	33,181 ± 2339	24,041 ± 2732	19,994 ± 7213	3968 ± 1823
Empididae	62 ± 41	42 ± 16	81 ± 23	68 ± 32	55 ± 13	63 ± 20	137 ± 97	52 ± 76	2 ± 3
Limoniidae	1526 ± 1805	394 ± 271	435 ± 313	332 ± 126	239 ± 129	392 ± 187	341 ± 87	84 ± 75	16 ± 15
Psychodidae	0 ± 0	0 ± 0	0 ± 0	6 ± 11	0 ± 0	0 ± 0	0 ± 0	6 ± 10	14 ± 16
Simuliidae	0 ± 0	15 ± 17	6 ± 5	2 ± 3	0 ± 0	141 ± 197	51 ± 50	2 ± 4	2 ± 3

[a] Density of pupae in parenthesis.

the exception of the pollution-tolerant Diptera *Psychodidae*, which colonized channels receiving 15% and 30% effluent by the end of the experiment. Trichoptera taxa tended to decrease in density and the treatment appeared to trigger reproduction in several taxa, including *Hydroptilidae* and *Rhyacophilidae*, indicated by the presence of pupae in higher densities at higher effluent concentrations (Table 2).

The diagram of the first PRC illustrates the multivariate invertebrate response to the experimental treatment, and represents the concentration-dependent deviations in community assemblages from the control over time (Fig. 2). The response pattern in the first PRC axis was significant ($p = 0.002$) and captured 34.4% of the total variance explained by the treatment regime. Differences between the invertebrate community in the treatment and the control groups increased with higher effluent concentrations and over time. Differences among treatments accounted for 23.5% of all variance in the invertebrate data, while differences in sampling times accounted from 16% of all variance. The second PRC axis was not significant and is not presented.

The invertebrate community at week 0 (April 14, 2009) represent common samples collected from the river and thus have an identical score of 0 on the PRC axis (Fig. 2). There was evidence of deviation of the treatment groups from the controls after one week of exposure, based on Monte Carlo permutation tests ($p = 0.008$). Post hoc Dunnett's tests indicated that the assemblages exposed to the highest concentration treatment (30% effluent) were significantly different than control ($p = 0.006$; Fig. 2). By the second week, treatment groups deviated further from the control and exhibited a clear dose-effect response, whereby differences between treatment and control groups increased with increasing effluent concentration. The treatment effects in the second sampling date were significant (Monte Carlo permutation test; $p = 0.002$) and post hoc tests between treatment and control groups indicate that the invertebrate communities exposed to 15% and 30% concentrations of effluent were significantly different from the controls (Dunnett's test; $p < 0.001$ for both comparisons).

The species weight diagram (Fig. 2) describes how the response of particular invertebrate taxa to the treatment relates to the overall response pattern indicated by the PRC diagram. Taxa indicated with positive species weights are thus expected to increase in abundance with higher treatment concentrations, relative to the controls, whereas taxa with negative species weights are expected to decrease in abundance. For example, the Diptera *Psychodidae* had the highest positive species weight (0.77) of all taxa, which reflects the colonization of this pollution-tolerant taxa only in channels receiving the highest level of effluent discharge (Table 2). In contrast, most taxa had negative species weights, including most of the Trichoptera, Ephemeroptera, and Diptera taxa, indicating a decrease in abundance in response to the exposure to effluent. However, the species weights for several taxa were close to 0 (e.g., *Rhyacophilidae* and *Ceratopogonidae*), indicating either a weak effect of the effluent treatment or a unique treatment response that did not closely follow the trajectory of the PRC curve.

3.2. Relationships of physical–chemical parameters to effluent treatments

The PRC diagram of the physical–chemical parameters reflects key differences in environmental conditions among the treatment groups (Fig. 3). The first PRC explained 72.3% in the total variance of the physical–chemical data, 66.5% of which was explained by the treatment regime. Measured nutrient and water quality parameters were significantly related to the treatment effects captured by the first PRC ($p = 0.002$), with most nutrient levels increasing with

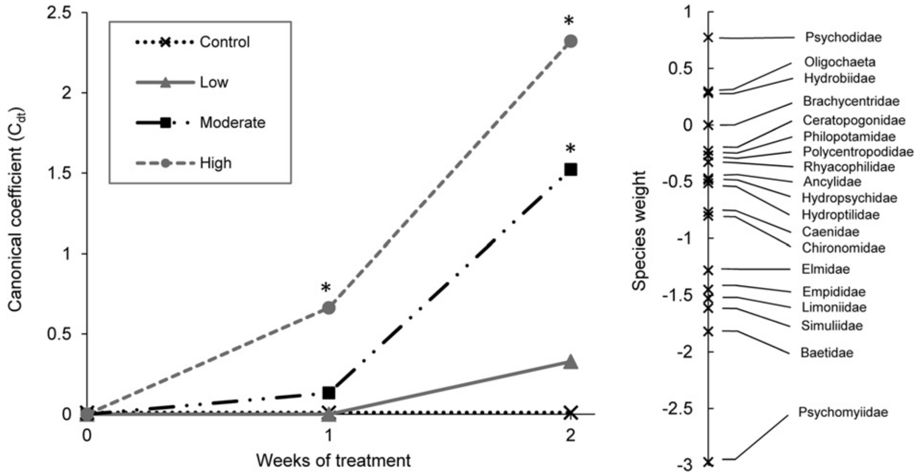

Fig. 2. Principal response curve (PRC) with species weights for the aquatic invertebrate community data, indicating the effects of exposure to 0% (control), 5% (low), 15% (moderate), and 30% (high) concentrations of treated wastewater. Asterisk (*) indicates significant ($p < 0.05$) differences between treatment and control groups.

higher effluent concentrations (Fig. 4). The second PRC was not statistically significant. The parameters most sensitive to treatment effects can be inferred from the species weights. The high species weights of ammonium, chlorine, sulfate, and phosphate reflects a strong correlation of these nutrients with effluent concentration, which is also captured by the high positive species weight of conductivity. In contrast, the negative species weights of dissolved oxygen indicate that oxygen availability decreased with increasing concentrations of effluent. The species weights of temperature, nitrite, and nitrate, were close to 0, indicating that these parameters were either insensitive to experimental treatments or showed a response to the treatment that is not reflected in the PRC diagram.

3.3. *Effects of effluent treatment on ecological quality status*

A total of 15 taxa were observed on the cobbles collected from the river at the beginning of the experiment, which is representative of the unimpaired reach of the Llobregat River upstream of the WWTP (Prat and Rieradevall, 2006). Half of the taxa were Ephemeroptera, Plecoptera, and Trichoptera (EPT), including many pollution-sensitive taxa in high abundance. Both the qualitative (IMWBP) and quantitative (IMMi-t) indices indicate that ecological status of the site is high or very high, following the classification thresholds defined by Munné and Prat (2009). The wastewater treatments had negative effects on biological quality indices (Table 3). After one weak of exposure to wastewater effluents, indices of community diversity and biological quality remained relatively stable in the control, low and moderate treatment groups (Table 3). However, the high treatment group had a much lower Shannon Diversity score, fewer total taxa, fewer EPT taxa, and lower values of log (SelEPTCD + 1). Based on the biotic quality indices, the ecological status of the 30% effluent treatment group was 'moderate', while the other treatment groups were in 'good' condition. By week two of the experiment, the low (5% effluent) treatment group had slightly lower values for most invertebrate community metrics, including the number of taxa, EPT taxa, IASPT, and log (SelEPTCD + 1). However, the biotic quality index values remained high enough to classify the invertebrate community in 'good' condition. The 15% treatment group had an intermediate effect on biological condition relative to the 5% and 30% effluent treatments after 2 weeks, indicated by the reduction in ecological status to 'moderate' condition. The channels receiving the high

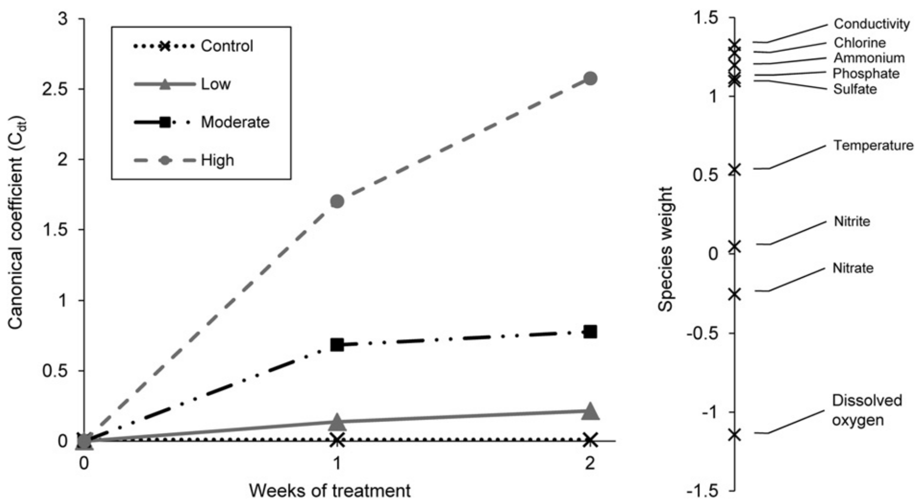

Fig. 3. Principal response curve (PRC) with species weights for the physicochemical parameter data, describing the relationship between physicochemical parameters and wastewater effluent at 0% (control), 5% (low), 15% (moderate), and 30% (high) concentration.

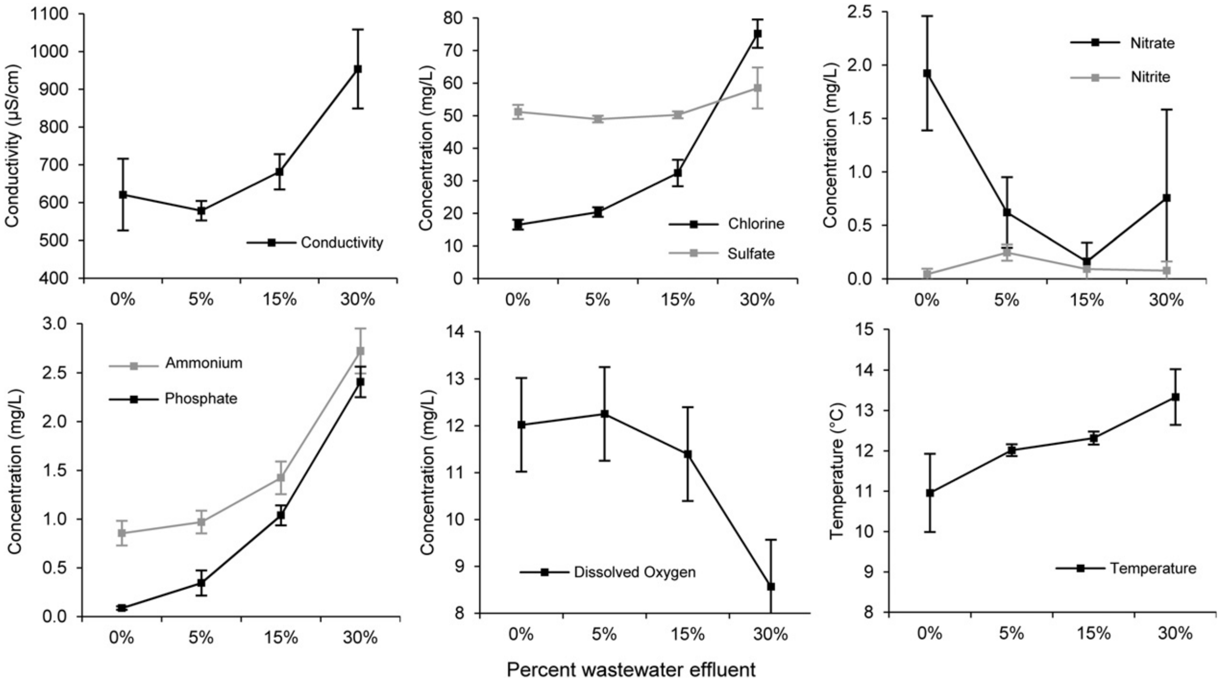

Fig. 4. Measured values (mean ± SD) of physicochemical parameters at different concentrations of treated waste water effluent.

(30%) treatment of sewage water declined to 'very poor' conditions by week two. Only 3 EPT taxa were present in the high-concentration treatment group after 2 weeks and most pollution-intolerant taxa were absent. The disappearance of sensitive species was concomitant with the increase of ammonia and chloride concentrations, which are known to be key physicochemical parameters that limit the presence of many aquatic invertebrate taxa in polluted rivers.

4. Discussion

The findings indicate that exposure to treated wastewater effluent has a significant impact on the composition and diversity of the aquatic invertebrate community. The changes in invertebrate assemblages appeared to follow a dose-dependent response, whereby treatment effects on the invertebrate community increased with elevated effluent concentrations (i.e., decreased dilution) and extended periods of exposure. The stream mesocosm channels receiving the highest concentration of effluent had the most pronounced shifts in the invertebrate community, characterized by a general decline in invertebrate densities and loss of several pollution-sensitive taxa. However, there was also evidence that exposure to 5% effluent discharges caused moderate impacts to the invertebrate community, including the loss of sensitive species and decline in several diversity metrics, suggesting that even low concentrations of effluent have a biologically important effect. The WWTP effluents used in the mesocosm contained elevated levels of ammonium, chloride, soluble phosphate, and sulfate that increased in response to the concentration of effluent in each treatment. Excessive nutrient loading has been shown to be the dominant cause of stream ecosystem impacts from WWTP discharges (e.g., Kosmala et al., 1999; Carey and Migliaccio, 2009; Martí et al., 2010) and is probably responsible for the observed changes in invertebrate assemblages in this study.

The changes in the abundance and composition of invertebrates led to a reduction in the ecological status of the stream mesocosm community. All of the community metrics and biotic indices showed a clear negative response to the wastewater treatments. The response was both treatment and time dependent; the higher the effluent concentration and the exposure time, the greater the reduction in the ecological status. These results demonstrate the utility of aquatic invertebrates for detecting wastewater pollution impacts and highlight the value of sampling invertebrates for assessing the biotic integrity of rivers (Metcalfe, 1989; Bonada et al.,

Table 3
Aquatic invertebrate community metrics and corresponding ecological status for Mediterranean rivers in Spain.

Treatment	Week	Diversity (H')	Number taxa (S)	EPT taxa	IASPT	Log (Sel EPTCD + 1)	IMMi-t	IBMWP	Ecological status[a]
River Control	0	1.26	15	8	5.3	3.08	0.96	0.59	very good, good
Control	1	1.08	14	7	4.7	2.8	0.83	0.48	good, moderate
Low	1	1.02	16	7	4.8	2.81	0.85	0.56	good, good
Moderate	1	0.72	16	7	4.7	2.63	0.83	0.55	good, good
High	1	0.49	13	6	4.6	1.88	0.72	0.44	moderate, moderate
Control	2	0.72	17	8	4.9	2.7	0.89	0.61	good, good
Low	2	0.77	15	7	4.6	2.33	0.79	0.51	good, good
Moderate	2	0.71	15	6	4.4	1.16	0.64	0.48	moderate, moderate
High	2	0.73	11	3	4.1	0	0.42	0.33	poor, poor

[a] Ecological status classification based on biotic index (IMMi-t, IBMWP) threshold values for Mediterranean rivers, developed by Munné and Prat (2009).

2006; Furse et al., 2006). Nevertheless, additional studies are needed to better understand how effluent discharges potentially affect invertebrate communities. The present experiment focused on the impacts of wastewater effluent at steady concentrations for a two week period. Future studies should evaluate how invertebrates respond to pulse impacts of effluent discharge, which often occur at WWTPs from overflows and spills during rain effects (Rueda et al., 2002; Canobbio et al., 2009). Further examination of the effects of wastewater effluent at different levels of biological organization, including traits, taxa, assemblages, and food webs, is also needed.

Observed community response patterns to the experimental treatments indicate that stream mesocosms are an effective method for investigating the effects of treated wastewater effluent on aquatic invertebrates. Although the artificial stream channels used in our mesocosm facility represent a simplified, less dynamic physical environment than the natural river bed, several aspects of the mesocosm design likely improved the 'realism' of the model system. For example, the use of an open, flow-through system facilitated passive invertebrate colonization and helped to avoid water quality problems often associated with recycled-water systems (Ledger et al., 2009). In addition, the close proximity of the mesocosm to the river of origin and minimal relocation disturbance of river cobbles were important for maintaining the original composition of the stream invertebrate community. The slightly higher taxa diversity and IASPT values (indicating a greater proportion of sensitive species) of the initial sample of river cobbles indicate that the mesocosm did have some effect on invertebrate assemblages. Nevertheless, the stability of invertebrate community metrics in the control treatments, evidenced by the high number of total and EPT taxa and biotic quality index ratings of 'good' status, indicates that mesocosm effects did not influence the observed responses to the effluent treatments. The consistency of our findings with field sampling from above and below the WWTP outfall (Caus and Prat, 2010) provides further evidence that invertebrate assemblages in the mesocosm responded in a similar manner as *in situ* communities to wastewater effluent discharges.

In contrast to field sampling approaches, mesocosms make it possible to isolate the effects of individual treatment factors and test potential causal relationships between treatments and biological responses. This is critical for the study of freshwater ecosystems, and Mediterranean rivers in particular, which are subject to multiple physical and chemical stressors (Ormerod et al., 2010; Ricart et al., 2010). Where rivers are impaired by multiple stressors, biomonitoring approaches may not be effective for detecting specific effects. For example, Gücker et al. (2006) concluded that the failure to identify adverse effects of wastewater effluents on aquatic macroinvertebrates was due to the fact that the community was already impoverished by the deterioration of water quality and habitat conditions. Similarly, Prat et al. (in review) found that discharges of reclaimed water had no effects on macroinvertebrate assemblages in a highly impacted reach of the lower Llobregat River because the community was already dominated by taxa tolerant to pollution. Therefore, mesocosms offer a useful and complementary approach to biomonitoring studies for quantifying the effects of pollution and other stressors on freshwater ecosystems.

In conclusion, this study confirms that treated wastewater effluent discharges have a significant impact on stream macroinvertebrate assemblages. The findings are consistent with the environmental assessment conducted by the Catalan Water Agency, which reported that WTTP are a dominant pressure on aquatic ecosystems and are preventing up to 40% of water bodies in the region from achieving good ecological status (Prat et al., in review). The preliminary results of this study suggest that WWTP effluent releases should probably be maintained at a dilution rate below 5% of the total discharge of the receiving waters to preserve healthy biotic assemblages. Further research is needed to more fully understand the short- and long-term impacts of WWTP effluent discharges on aquatic organisms. Mesocosm experiments can play an important role in supplementing the knowledge gained from biomonitoring programs and ultimately improving the management and conservation of freshwater ecosystems.

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References

Belanger, S.E., Lee, D.M., Bowling, J.W., LeBlanc, E.M., 2004. Responses of periphyton and invertebrates to a tetradecyl-pentadecyl sulfate mixture in stream mesocosms. Environmental Toxicology and Chemistry 23, 2202–2213.

Bonada, N., Prat, N., Resh, V.H., Statzner, B., 2006. Developments in aquatic insect biomonitoring: a comparative analysis of recent approaches. Annual Review of Entomology 51, 495–523.

Bond, N.R., Downes, B.J., 2003. The independent and interactive effects of fine sediment and flow on benthic invertebrate communities characteristic of small upland streams. Freshwater Biology 48, 455–465.

Brooks, B.W., Stanley, J.K., White, J.C., Turner, P.K., Wu, K.B., La Point, T.W., 2004. Laboratory and field responses to cadmium: an experimental study in effluent-dominated stream mesocosms. Environmental Toxicology and Chemistry 23, 1057–1064.

Brown, C.J.M., Knight, B.W., McMaster, M.E., Munkittrick, K.R., Oakes, K.D., Tetreault, G.R., Servos, M.R., 2011. The effects of tertiary treated municipal wastewater on fish communities of a small river tributary in southern Ontario, Canada. Environmental Pollution 159, 1923–1931.

Canobbio, S., Mezzanotte, V., Sanfilippo, U., Benvenuto, F., 2009. Effect of multiple stressors on water quality and macroinvertebrate assemblages in an effluent-dominated stream. Water, Air, & Soil Pollution 198, 359–371.

Cao, Y., Bark, A.W., Williams, W.P., 1996. Measuring the responses of macroinvertebrate communities to water pollution: a comparison of multivariate approaches, biotic and diversity indices. Hydrobiologia 341, 1–19.

Carey, R.O., Migliaccio, K.W., 2009. Contribution of wastewater treatment plant effluents to nutrient dynamics in aquatic systems: a review. Environmental Management 44, 205–217.

Caus, M., Prat, N., 2010. Estudi de la qualitat de l'aigua del riu Llobregat i afluents pròxims als runams salins del Bages. M.S.C. report. Universitat de Barcelona, Department d'Ecologia, Barcelona, Spain.

Coimbra, C.N., Graça, M.A.S., Cortés, R.M., 1996. The effects of a basic effluent on macroinvertebrate community structure in a temporary Mediterranean river. Environmental Pollution 94, 301–307.

Colville, A., Jones, P., Pablo, F., Krassoi, F., Hose, G., Lim, R., 2008. Effects of chlorpyrifos on macroinvertebrate communities in coastal stream mesocosms. Ecotoxicology 17, 173–180.

Dorn, P.B., Rodgers, J.H., Gillespie, W.B., Lizotte, R.E., Dunn, A.W., 1997. The effects of a C12–13 linear alcohol ethoxylate surfactant on periphyton, macrophytes, invertebrates and fish in stream mesocosms. Environmental Toxicology and Chemistry 16, 1634–1645.

Duarte, S., Pascoal, C., Alves, A., Correia, A., Cássio, F., 2008. Copper and zinc mixtures induce shifts in microbial communities and reduce leaf litter decomposition in streams. Freshwater Biology 53, 91–101.

Dyer, S.D., Wang, X.H., 2002. A comparison of stream biological responses to discharge from wastewater treatment plants in high and low population density areas. Environmental Toxicology and Chemistry 21, 1065–1075.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Leveque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81, 163–182.

Furse, M., Hering, D., Moog, O., Verdonschot, P., Johnson, R., Brabec, K., Gritzalis, K., Buffagni, A., Pinto, P., Friberg, N., Murray-Bligh, J., Kokes, J., Alber, R., Usseglio-Polatera, P., Haase, P., Sweeting, R., Bis, B., Szoszkiewicz, K., Soszka, H., Springe, G., Sporka, F., Krno, I., 2006. The STAR project: context, objectives and approaches. Hydrobiologia 566, 3–29.

Gafny, S., Goren, M., Gasith, A., 2000. Habitat condition and fish assemblage structure in a coastal mediterranean stream (Yarqon, Israel) receiving domestic effluent. Hydrobiologia 422–423, 319–330.

Garric, J., Voilat, B., Nguyen, D.K., Bray, M., Migeon, B., Kosmala, A., 1996. Ecotoxicological and chemical characterization of municipal wastewater treatment plant effluents. Water Science and Technology 33, 83–91.
Gasith, A., Resh, V.H., 1999. Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. Annual Review of Ecology and Systematics 30, 51–81.
Gücker, B., Brauns, M., Pusch, M.T., 2006. Effects of wastewater treatment plant discharge on ecosystem structure and function of lowland streams. Journal of the North American Benthological Society 25, 313–329.
Hickey, C.W., Golding, L.A., 2002. Response of macroinvertebrates to copper and zinc in a stream mesocosm. Environmental Toxicology and Chemistry 21, 1854–1863.
Hruska, K.A., Dubé, M.G., 2004. Using artificial streams to assess the effects of metal-mining effluent on the life cycle of the freshwater midge (*Chironomustentans*) in situ. Environmental Toxicology and Chemistry 23, 2709–2718.
Kosmala, A., Charvet, S., Roger, M.C., Faessel, B., 1999. Impact assessment of a wastewater treatment plant effluent using instream invertebrates and the *Ceriodaphniadubia* chronic toxicity test. Water Research 33, 266–278.
Kümmerer, K., 2009. Antibiotics in the aquatic environment – a review – Part II. Chemosphere 75, 435–441.
Lamberti, G.A., Steinman, A.D., 1993. Research in artificial streams - applications, uses, and abuses. Journal of the North American Benthological Society 12, 313–384.
Ledger, M.E., Harris, R.M.L., Milner, A.M.M., Armitage, P.D., 2006. Disturbance, biological legacies and community development in stream mesocosms. Oecologia 148, 682–691.
Ledger, M.E., Harris, R.M.L., Armitage, P.D., Milner, A.M., 2009. Realism of model ecosystems: an evaluation of physicochemistry and macroinvertebrate assemblages in artificial streams. Hydrobiologia 617, 91–99.
Martí, E., Aumatell, J., Gode, L., 2004. Nutrient retention efficiency in streams receiving inputs from wastewater treatment plants. Journal of Environmental Quality 33, 285–293.
Martí, E., Riera, J.L., Sabater, F., 2010. Effects of wastewater treatment plants on stream nutrient dynamics under water scarcity conditions. In: Sabater, S., Barceló, D. (Eds.), Water Scarcity in the Mediterranean: Perspectives under Global Climate Change. Springer, Berlin/Heidelberg, pp. 173–195.
Masseret, E., Amblard, C., Bourdier, G., 1998. Changes in the structure and metabolic activities of periphytic communities in a stream receiving treated sewage from a waste stabilization pond. Water Research 32, 2299–2314.
Metcalfe, J.L., 1989. Biological water quality assessment of running waters based on macroinvertebrate communities: History and present status in Europe. Environmental Pollution 60, 101–139.
Meyer, J., Bester, K., 2004. Organophosphate flame retardants and plasticisers in wastewater treatment plants. Journal of Environmental Monitoring 6, 599–605.
Munné, A., Prat, N., 2009. Use of macroinvertebrate-based multimetric indices for water quality evaluation in Spanish Mediterranean rivers: an intercalibration approach with the IBMWP index. Hydrobiologia 628, 203–225.
Odum, E.P., 1984. The mesocosm. BioScience 34, 558–562.
Ormerod, S.J., Dobson, M., Hildrew, A.G., Townsend, C.R., 2010. Multiple stressors in freshwater ecosystems. Freshwater Biology 55, 1–4.
Ortíz, J.D., Puig, M.A., 2007. Point source effects on density, biomass and diversity of benthic macroinvertebrates in a Mediterranean stream. River Research and Applications 23, 155–170.
Paxéus, N., 1996. Organic pollutants in the effluents of large wastewater treatment plants in Sweden. Water Research 30, 1115–1122.
Pearson, R.G., Connolly, N.M., 2000. Nutrient enhancement, food quality and community dynamics in a tropical rainforest stream. Freshwater Biology 43, 31–42.
Perrin, C.J., Wilkes, B., Richardson, J.S., 1992. Stream periphyton and benthic insect responses to additions of treated acid mine drainage in a continuous-flow onsite mesocosm. Environmental Toxicology and Chemistry 11, 1513–1525.
Petersen, J.E., Englund, G., 2005. Dimensional approaches to designing better experimental ecosystems: a practitioners guide with examples. Oecologia 145, 216–224.
Pinto, A.L., Varandas, S., Coimbra, A.M., Carrola, J., Fontainhas-Fernandes, A., 2010. Mullet and gudgeon liver histopathology and macroinvertebrate indexes and metrics upstream and downstream from a wastewater treatment plant (Febros River-Portugal). Environmental Monitoring and Assessment 169, 569–585.
Postel, S.L., 2007. Aquatic ecosystem protection and drinking water utilities. American Water Works Association Journal 99, 52–63.
Postel, S., Carpenter, S., 1997. Freshwater ecosystem services. In: Daily, G. (Ed.), Nature's Services: Societal Dependence of Natural Ecosystems. Island Press, Washington D.C., pp. 195–215.
Prat, N., Munné, A., 2000. Water use and quality and stream flow in a Mediterranean stream. Water Research 34, 3876–3881.
Prat, N., Rieradevall, M., 2006. 25-years of biomonitoring in two Mediterranean streams (Llobregat and Besos basins. NE spain). Limnetica 25, 541–550.
Prat, N., Ward, J.V., 1994. The tamed river. In: Margalef, R. (Ed.), Limnology Now: A Paradigm of Planatery Problems. Elsevier Science, New York, pp. 219–236.
in reviewPrat, N., Rieradevall, M., Barata, C., Munné, A., in review. Assessing the effects of tertiary treated wastewater reuse on a Mediterreanean river (Llobregat, NE Spain). Part IV: macroinvertebrate community assemblages, biomarkers and ecological status. Environmental Science and Pollution Research.
Prat, N., Puig, M.A., González, G., Tort, M.J., Estrada, M., 1984. The Llobregat: a Mediterranean river fed by the pyrenées. In: Whitton, B.A. (Ed.), Ecology of European Rivers. Blackwell, London, pp. 527–552.
Ricart, M., Guasch, H., Barceló, D., Brix, R., Conceição, M.H., Geiszinger, A., Alda, M.J.L.d., López-Doval, J.C., Muñoz, I., Postigo, C., Romaní, A.M., Villagrasa, M., Sabater, S., 2010. Primary and complex stressors in polluted Mediterranean rivers: Pesticide effects on biological communities. Journal of Hydrology 383, 52–61.
Richardson, J.S., Kiffney, P.M., 2000. Responses of a macroinvertebrate community from a pristine, southern British Columbia, Canada, stream to metals in experimental mesocosms. Environmental Toxicology and Chemistry 19, 736–743.
Richardson, J.S., Perrin, C.J., 1994. Effects of the bacterial insecticide *Bacillus thuringiensisvarKurstki* (*Btk*) on a stream benthic community. Canadian Journal of Fisheries and Aquatic Sciences, 1037–1045.
Rueda, J., Camacho, A., Mezquita, F., Hernández, R., Roca, J.R., 2002. Effect of episodic and regular sewage discharges on the water chemistry and macroinvertebrate fauna of a Mediterranean stream. Water, Air, & Soil Pollution 140, 425–444.
Ruggiero, A., Solimini, A.G., Carchini, G., 2006. Effects of a waste water treatment plant on organic matter dynamics and ecosystem functioning in a mediterranean stream. Annales De Limnologie-International Journal of Limnology 42, 97–107.
Smith, V.H., Tilman, G.D., Nekola, J.C., 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environmental Pollution 100, 179–196.
Ter Braak C.J.F., Šmilauer P, 2002. CANOCO version 4.5. Biometrics – plant research International, Wageningen, the Netherlands.
Van den Brink, P.J., TerBraak, C.J.F., 1999. Principal response curves: analysis of time-dependent multivariate responses of biological community to stress. Environmental Toxicology and Chemistry 18, 138–148.
Van den Brink, P.J., Van Wijngaarden, R.P.A., Lucassen, W.G.H., Brock, T.C.M., Leeuwangh, P., 1996. Effects of the insecticide Dursban 4E (active ingredient chlorpyrifos) in outdoor experimental ditches: II. Invertebrate community responses and recovery. Environmental Toxicology and Chemistry 15, 1143–1153.
Van Damme, P.A., Hamel, C., Ayala, A., Bervoets, L., 2008. Macroinvertebrate community response to acid mine drainage in rivers of the high Andes (Bolivia). Environmental Pollution 156, 1061–1068.