



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

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### ABSTRACT

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.

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### 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 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

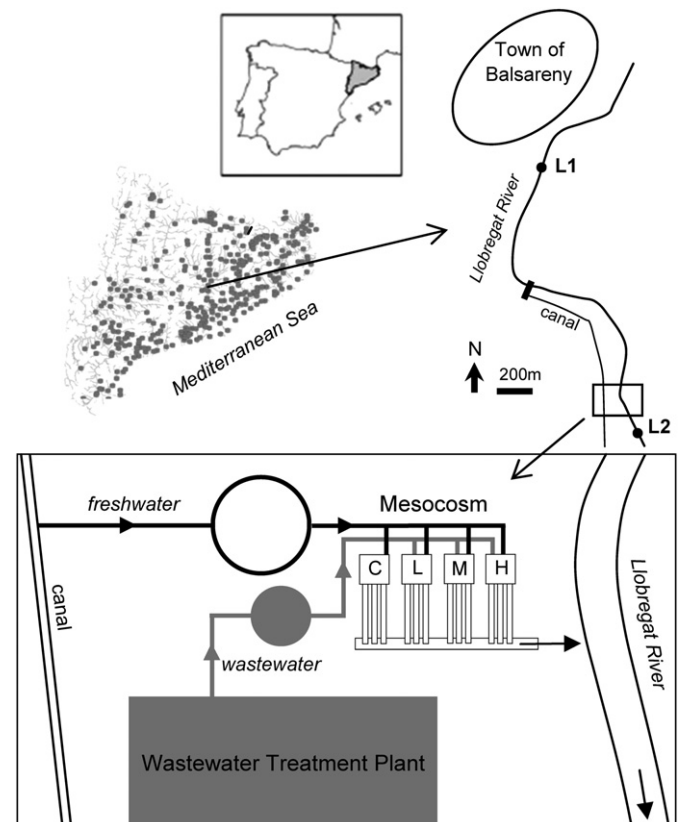
The stream mesocosm experiment was conducted at a wastewater treatment plant near the town of Balsareny (41°50′54″N, 1°52′49″E), located approximately

60 km north of Barcelona, Spain (Fig. 1). The Balsareny WWTP serves a population of 3410. Approximately 700 m<sup>3</sup> 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 Balsareny 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.

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