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Diatom responses to sewage inputs and hydrological alteration in Mediterranean streams☆

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We analyzed the conjoint effects of sewage inputs and hydrological alteration on the occurrence of teratological forms and on the assemblage composition of stream benthic diatoms. The study was performed in 11 Mediterranean streams which received treated or untreated urban sewage (Impact sites, I), whose composition and morphological anomalies were compared to upstream unaffected (Control, C) sites. The impact sites had high concentrations of ammonium, phosphorus, and pharmaceutical compounds (antibiotics, analgesics, and anti-inflammatories), particularly in those receiving untreated sewage. Impact sites had a higher proportion of teratological forms as well as a prevalence of diatom taxa tolerant to pollution. The differences in the diatom assemblage composition between the paired C and I sites were the largest in the impacted sites that received untreated sewage inputs as well as in the systems with lower dilution capacity. In these sites, the diatom assemblage was composed by a few pollution-tolerant species. Mediterranean river systems facing hydrological stress are highly sensitive to chemical contamination, leading to the homogenization of their diatom assemblages.

1. Introduction

Algal assemblages in rivers are potentially affected by multiple stressors, including chemical contamination, irradiance excess and high water temperatures, as well as hydrological alterations. Mediterranean streams are naturally characterized by periods of hydrological stability, only interrupted by autumnal floods and intermittency during the summer period (Lake, 2003; Sabater and Tockner, 2010). Most climate change scenarios predict low-flow waters to become more pronounced in the next century in the Mediterranean region (Giorgi and Lionello, 2008). Further, rising human exploitation of water resources directly affects river ecosystems (Alcamo et al., 2007). As a result, streams and rivers show temporal and spatial increases of hydrological stability (i.e. more extended and frequent basal flow periods) and even non-natural loss of flowing water or streambed desiccation.

Non-natural conditions of low flow and hydrological stability in human-impacted Mediterranean rivers may be associated to higher concentrations of nutrients, organic matter (Almeida et al., 2014) and organic micropollutants (Sabater et al., 2016). Wastewater discharges of urban origin reach freshwater systems either via wastewater treatment plants (WWTP) or through direct sewage inputs. Wastewaters carry pharmaceutically active compounds (PhACs) together with other micro-contaminants, and organic matter (Gros et al., 2007; Muñoz et al., 2009). These may reach potentially hazardous concentrations (Gros et al., 2007) when entering watercourses with reduced dilution capacity.

The co-occurring chemical stressors and hydrological alterations in Mediterranean river systems produce a conjoint pressure on biological assemblages. Amongst them, the diatoms are suitable ecological indicators of the effects of these stressors, given their high sensitivity to chemical and physical conditions (Pan et al., 1999). Diatoms are siliceous algae which make up the largest fraction of algal assemblages colonizing the streambed. Diatoms may be affected morphologically by different sources of stress, producing teratologies (Cantonati et al., 2014), and their assemblages respond to the stressors by shifting on their composition and relative abundances (Tornés et al., 2007; Sabater et al., 2016). The diatom taxa show specific tolerances to stressors, some (e.g.

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Simonsenia delognei) being able to face harsh conditions during desiccation (Souffreau et al., 2013; Novais et al., 2014; Falasco et al., 2016) and to acclimate to unfavorable hydric conditions (Souffreau et al., 2013), while many others have defined sensitivities to chemical contamination due to nutrients, organic matter and organic micropollutants (e.g. *Nitzschia palea*, *Navicula gregaria*; Tornés et al., 2007; Sabater et al., 2016).

While the separate effects of hydrological and chemical stress on diatom assemblages have been well studied (e.g. Muñoz et al., 2009; Barthès et al., 2014; Teittinen et al., 2015; Piano et al., 2017), their response to the two conjoint stressors is still uncertain (but see Corcoll et al., 2015; Ponsatí et al., 2016). We here analyzed the combined effects of sewage inputs and hydrological alteration on stream benthic diatoms and non-siliceous algae in small and medium-sized Mediterranean rivers. We selected 11 streams receiving treated or untreated sewage discharges from urban point sources. The streams differed on the received chemical impact as well as on their dilution capacity.

We assumed that the potential chemical impact on the diatom assemblages would be a function of the dilution capacity of the receiving system as well as of the type of sewage entering each of the systems. We performed this analysis by comparing the occurrence of teratologic forms and the compositional change of diatom assemblages in the impact sites with respect to their respective upstream (control) sites. We hypothesized (i) that chemical pollution would drive the changes in morphology and taxonomic composition to the downstream sites, (ii) that the lower the dilution capacity of the receiving system, the higher the changes in diatom composition, and (iii) that the most affected impact sites will converge in the composition of their diatom assemblages by favoring the dominance of the pollution-tolerant taxa.

2. Material and methods

2.1. Study sites

The study was conducted in 11 Mediterranean streams tributaries from the lower part of the Ebro River (NE Iberian Peninsula) (Fig. 1). The streams were comparable in terms of geology (mostly calcareous), and ranged between orders 2 to 4. All the sites shared a similar light regime, all being of small to medium order with sparse canopy cover. Most of the rivers were poorly urbanized, mostly having forested and agricultural land uses in their basins. All of them receive strong pressure for the use of their water resources, in the forms of direct water abstraction or groundwater exploitation. We selected two sites (i.e. Control-upstream and Impact-downstream) in each of these systems. The Control (C) sites did not receive direct inputs from human activities in its vicinity, but were submitted to hydrological alteration because of the intensive use of water resources. The Impact (I) sites were immediately downstream to wastewater discharges from urban sources (small cities ranging 540–7000 inhabitants), and were submitted to analogous hydrological alterations than those affecting the C site. The distance between the C and I sites ranged from a few hundred meters in the smaller systems to a few kilometers in the larger systems. The sites were selected not to have tributaries or dams entering or intercepting the stream in between the C and I sites. Some of the sites received treated effluents from WWTP while others directly received untreated urban sewage (Table 1). In the smaller rivers (having the lowest water flow) the sewage outflow represented a moderate increase to the basal water flow but in most of the sites the change was inappreciable (Table 1).

2.2. Physical and chemical measurements

We conducted sampling surveys during early summer (June) of 2015 and spring (April) of 2016. These two sampling periods respectively covered lower-water flow (summer) and higher-water flow (spring) conditions. Water depth, velocity, and instant discharge were measured at each sampling campaign with an acoustic Doppler velocity meter (ADV; Flow Tracker, SonTek Handheld-AD®, P-4077). Water pH, dissolved oxygen, and electrical conductivity were measured *in situ* using hand-held probes at each sampling campaign (WTW, Weilheim, Germany). One water sample for nutrient analyses (nitrate (NO_3^-, $\mu g\ N \cdot L^{-1}$), nitrite (NO_2^-, $\mu g\ N \cdot L^{-1}$), ammonium (NH_4^+, $\mu g\ N \cdot L^{-1}$) and phosphate (PO_4^{3-}, $\mu g\ P \cdot L^{-1}$)), and dissolved organic carbon (DOC, $mg \cdot L^{-1}$) were collected at each site, filtered in 0.7 μm GF/F filters (Whatman Int. Ltd., Maidstone, UK) and kept at −20 °C until analysis. Phosphate concentration was determined colorimetrically using a spectrophotometer (Alliance-AMS Smartchem 140, AMS, Frepillon, France), after Murphy and Riley, (Murphy and Riley, 1962). Nitrite, nitrate and ammonium concentrations were determined on a Dionex ICS-5000 ion chromatograph (Dionex Co., Sunnyvale, USA; Hach, 2002). DOC concentrations were determined on a Shimadzu TOC-V CSH coupled to a TNM-1 module (Shimadzu Co., Kyoto, Japan). DOC results were only available for the second sampling campaign, and therefore were not used in the statistical analyses (see below).

Pharmaceutical products were assumed to be the dominant microcontaminants given the urban sources of wastewaters. Their continuous discharge into the aquatic environment makes the PhACs pseudo-persistent contaminants, potentially able to cause adverse effects on living organisms and the environment (Daughton and Ternes, 1999). The PhACs analysis in water samples was conducted following the method developed by Gros et al. (2012). Briefly, the analyses were carried out with an off-line solid phase extraction (SPE) followed by ultra-high-performance liquid chromatography coupled to triple quadrupole linear ion trap tandem mass spectrometry (UHPLC-QqLIT-MS^2). Chromatographic separations were carried out with a Waters Acquity Ultra-Performance™ liquid chromatography system, coupled to a 5500 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer (Applied Biosystems, Foster City, CA, USA) with a turbo Ion Spray source. Quantification was carried out by isotope dilution. Finally, all data were acquired and processed using Analyst 1.5.1 software, while quantification was carried out by isotope dilution. Detailed information regarding chemicals and reagents used, as well as method performance parameters of target compounds including limits of detections (LODs), limits of quantifications (LOQs) and recovery rates are described in detail in Mandaric et al. (2018).

2.3. Algal (diatom) sampling and analysis

The algal collection was performed simultaneously to the physical and chemical measurements, in June 2015 and April 2016. For the algal collection, at least five stones were randomly collected from the stream bottom in riffle sections of the C and I sites. The substrata were scraped with a knife and a hard bristled toothbrush to fully detach the algal assemblage to a final area of ~50 cm^2. Samples were preserved in 4% formaldehyde until analysis. Each algal sample was partitioned in the laboratory for the taxonomic analysis of diatoms, and for the determination of non-diatom algae and cyanobacteria.

The non-diatom algal fraction was inspected under light microscopy (Nikon Eclipse 80i, Tokyo, Japan) at a magnification of 400×, after performing 50 random microscope fields per aliquot. Taxonomic determination was performed at the genus level and estimated after semi-quantitative analysis based on cell numbers