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Environmental stressors as a driver of the trait composition of benthic macroinvertebrate assemblages in polluted Iberian rivers

Maja Kuzmanovic[a,b,*], Sylvain Dolédec[c], Nuria de Castro-Catala[d], Antoni Ginebreda[a], Sergi Sabater[b,e], Isabel Muñoz[d], Damià Barceló[a,b]

[a] *Department of Environmental Chemistry, IDAEA-CSIC, C/Jordi Girona 18-26, 08034 Barcelona, Spain*
[b] *Catalan Institute for Water Research (ICRA), Parc Científic i Tecnològic de la Universitat de Girona, C/Emili Grahit, 101 Edifici H2O, 17003 Girona, Spain*
[c] *UMR 5023, LEHNA, Biodiversité et Plasticité dans les Hydrosystèmes, Université Lyon 1, 69100 Villeurbanne, France*
[d] *Department of Ecology, Universitat de Barcelona, Av. Diagonal, 643, 08028 Barcelona, Spain*
[e] *GRECO, Institut d'Ecologia Aquàtica, Universitat de Girona, Facultat de Ciències, Campus Montilivi, 17003 Girona, Spain*

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ABSTRACT

We used the trait composition of macroinvertebrate communities to identify the effects of pesticides and multiple stressors associated with urban land use at different sites of four rivers in Spain. Several physical and chemical stressors (high metal pollution, nutrients, elevated temperature and flow alterations) affected the urban sites. The occurrence of multiple stressors influenced aquatic assemblages at 50% of the sites. We hypothesized that the trait composition of macroinvertebrate assemblages would reflect the strategies that the assemblages used to cope with the respective environmental stressors. We used RLQ and fourth corner analysis to address the relationship between stressors and the trait composition of benthic macroinvertebrates. We found a statistically significant relationship between the trait composition and the exposure of assemblages to environmental stressors. The first RLQ dimension, which explained most of the variability, clearly separated sites according to the stressors. Urban-related stressors selected taxa that were mainly plurivoltine and fed on deposits. In contrast, pesticide impacted sites selected taxa with high levels of egg protection (better egg survival), indicating a potentially higher risk for egg mortality. Moreover, the trait diversity of assemblages at urban sites was low compared to that observed in pesticide impacted sites, suggesting the homogenization of assemblages in urban areas.

1. Introduction

River ecosystems are impacted by a variety of anthropogenic stressors (Vörösmarty et al., 2010) and changes in the taxonomic and functional diversity of local species are expected on the global scale (Olden et al., 2004). However, the successful quantification of the relationship between the occurrence of particular stressors and biological indicators across large geographical areas remains challenging. In addition, an increasing number of stressors are co-occurring and impact the biota simultaneously (Navarro-Ortega et al., 2015). Therefore, it is of utmost importance to disentangle the effects of co-occurring stressors, in order to determine which stressor should be given priority in river basin management. The growing human population and resulting land use changes from natural to urban and agricultural have increased pressure on river ecosystems. Agriculture and urbanization are recognized as being amongst the main causes of stream impairment (Paul and Meyer, 2001). Water and habitat quality are often degraded in the streams draining agricultural land (Allan, 2004) due to the increased input of pesticides, sediments and nutrients, as well as hydrological alterations due to water abstraction (Elbrecht et al., 2016; Tilman et al., 2002). Effects of pesticides on sensitive species have been observed in streams (e.g. Liess and Von Der Ohe, 2005; Schäfer et al., 2007) using trait based SPEAR index. In a recent study by Malaj et al. (2014), the scale of the problem was revealed, since it was estimated that organic pollutants, among which pesticides were the major contributors to the risk, threaten the health of freshwater ecosystems across the whole of Europe. Furthermore, in streams draining urban land, consistent ecological degradation also occurs (Walsh et al., 2005). Increasing run-off from impervious surfaces (i.e., asphalt, concrete or stone), input of storm water from piped drainage systems (Walsh et al., 2005) and wastewater discharges (Paul and Meyer, 2001) can cause drastic changes in urban streams. The symptoms generally associated with urbanization include "flashy" hydrograph, changes in channel morphology, high concentrations of

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[*] Correspondence to: Catalan Institute for Water Research (ICRA), Carrer Emili Grahit, 101, E-17003 Girona, Spain.
E-mail address: mkuzmanovic@icra.cat (M. Kuzmanovic).

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metals, nutrients and organic toxicants and elevated water temperature. These modifications generally result in the decline of sensitive species (Wenger et al., 2009) and changes in ecosystem processes such as nutrient uptake (Paul and Meyer, 2001).

Stream macroinvertebrates have long been used as indicators for water quality assessment (Rosenberg and Resh, 1992). However, natural variability and confounding factors can mask the effect of a particular stressor (Schäfer et al., 2007), especially over large geographical area. To overcome this problem, more attention has been given to the use of the biological traits of taxa such as generation time, body size, body form and dispersal ability (Statzner et al., 2005; Tachet et al., 2010; Usseglio-Polatera et al., 2000). These characteristics may be used to help interpret changes in assemblages across environmental gradients and to improve the robustness of traditional stream biomonitoring (Dolédec and Statzner, 2008). According to the habitat template theory (Southwood, 1977), the spatial and temporal characteristics of the habitat provide a framework against which species have evolved characteristic life-history strategies to maximize their fitness and survival (Poff, 1997; Townsend and Hildrew, 1994). Life-history strategies include different combinations of traits that represent the solution to a given ecological problem (Verberk et al., 2008). The use of multiple traits, described through multiple trait categories or states, has successfully discriminated between different stressors (Dolédec and Statzner, 2008; Dolédec et al., 1999; Mondy and Usseglio-Polatera, 2013). Multiple-trait based approaches have shown promise for biomonitoring because most stressors should affect only certain trait categories (Statzner et al., 2001, 2004, 2005), which can be useful for discriminating among multiple stressors. Furthermore, unlike species composition, which changes along geographical and downstream gradients, some traits are thought to vary little across temporal and spatial scales, which makes them useful for large-scale studies (Statzner et al., 2001, 2004, 2005).

In this study, we used invertebrate traits to discriminate between the different types of human impacts in several basins of the Iberian Peninsula. We selected 16 sampling sites from four Mediterranean river basins with known human pressures (pesticides, multiple urban stressors and mixed). We further selected species traits that were thought to specifically respond to these stressors. The aim was to test the ability of a multiple trait-based approach to show that traits were not randomly distributed across assemblages in studied rivers and that different trait combinations responded to specific conditions in relation to the environment (urban vs. pesticide impacted).

2. Materials and methods

2.1. Study area

The study area included four river basins located across the Mediterranean part of the Iberian Peninsula: the Ebro and Llobregat in the North-East, Júcar in the East and Guadalquivir in the South of the Peninsula (Fig. 1). A total of 16 sites were selected: four sites in the Ebro basin (coded E1, E2, E3 and E5), five sites in the Llobregat basin (L3, L4, L5, L6 and L7), five sites in the Júcar basin (J1, J2, J4, J5 and J6) and two sites in the Guadalquivir basin (G1 and G4). Each site receives a variety of diffuse and point source inputs depending on catchment land use (Fig. S1, Table S1, in Supplementary data). Some of the sites are located in urban areas; the other sites are located in areas where a high risk of pesticide toxicity has previously been reported (De Castro-Català et al., 2016; Kuzmanović et al., 2015a; López-Doval et al., 2012). The data used in this study were gathered within the SCARCE-CONSOLIDER project (Navarro-Ortega et al., 2012) in which the sampling for chemical and biological analyses was performed during the autumn of 2010.

2.2. Physical and chemical data

Organic pollutants were measured using analytical techniques based on gas chromatography-tandem mass spectrometry and liquid chromatography-tandem and hybrid mass spectrometry (Masiá et al., 2013; Osorio et al., 2014). To assess the toxic risk at each sampling site, toxic units (TU) were calculated using the measured concentrations of the compound (MEC) and respective acute toxicity data (EC50) for *Daphnia sp*. The sums of toxic units for each of the compound families ($TU_{pesticides}$ and TU_{metals} in Table 1) were calculated as the risk estimate posed by different groups of toxicants. The major contributors to the pesticide toxicity risk were insecticides (e.g., chlorpyriphos or chlorfenvinphos) whereas copper was the main contributor to the metal toxicity risk. More details on measurements of the chemical compounds and risk assessment associated with our study can be found in Kuzmanović et al. (2015a, 2015b). Other physical and chemical variables included average sediment particle size (Phimoy in Table 1) and variance (Phivar) at the Phi scale [range from -8 (boulder) to > 10 (colloid)], flow variations (expressed as a 3-month coefficient of variation (CV) prior to sampling), average precipitation (3-month average), water temperature (T), dissolved oxygen (O_2), dissolved organic carbon (DOC), conductivity, nutrients ($N\text{-}NO_3$, and $P\text{-}PO_4$), percentage of organic matter in sediment (OM) and the altitude of sampling sites. The OM content, toxic units and nutrient data were log-transformed prior to analysis. The catchment land use types were estimated from Corine Land Cover (2006) using Arc Map 10.1 software and the variable that synthesized naturalness was calculated as the weighted mean of three categories (Urban, Agricultural, Natural) arbitrarily weighted by a coefficient of 1, 5 and 100, respectively (LU in Table 1; see Supplementary material). Further details on chemical and physical data measurements are available in Sabater et al. (2016).

2.3. Site classification

We determined which stressors were present at sampling sites (Fig. 1) and according to the dominant stressor, sites were classified into three groups (pesticide impacted, urban and mixed). The pesticide impacted sites (E1, E5, J1, J2, J4, J5, J6, G1) where those where acute risk was posed by pesticides ($\log TU > -1$, (Fig. S2, Kuzmanović et al., 2015b)). Sites classified as urban (L3, L4, L5, L6, L7, and G4) were those impacted by other stressors (e.g., metals, nutrients, elevated temperature, low oxygen level, Fig. 1) which were all highly correlated to urban land use (Table S2, Supplementary). At urban sites, the risk of pesticide toxicity was below acute levels. Finally, two sites were classified as mixed (E2 and E3) because they were affected both by pesticides and multiple stressors related to urban land use. Whether an environmental variable can be considered a stressor was evaluated on the basis of thresholds derived from legislation or the literature (Table S3).

2.4. Macroinvertebrate sampling

At each site, five sediment samples were randomly collected using a polyvinyl sand corer (24 cm^2 area). Each sample was sieved through a 500-µm mesh and fixed with 4% formaldehyde. Macroinvertebrates were sorted, counted and identified in the laboratory under a dissecting microscope (Leica Stereomicroscope). *Chironomidae* were identified at the genus level, while almost all other taxa were identified at the species level (list of taxa available in Supplementary material). Abundances were referred to on the basis of sediment surface area (De Castro-Català et al., 2015).

2.5. Biological traits

Traits were derived from a European database compiled by Tachet et al. (2010) and completed for Mediterranean taxa by Bonada et al. (Bonada and Dolédec, 2011; Bonada et al., 2007). In this database, the