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Multiple stressors in Mediterranean freshwater ecosystems: The Llobregat River as a paradigm

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Summary. Hydrological modifications drive other ecological stressors of freshwater ecosystems and interact with them. The present paper examines the relevance of hydrological disturbances resulting from global change by presenting the case of the Llobregat River, a highly disturbed system in NE Spain. The Llobregat is a clear example of a Mediterranean river suffering from multiple stressors. Both the distribution and abundance of organisms and ecosystem functioning as a whole are greatly determined by water scarcity, water salinity, nutrient concentration, and organic (and inorganic) pollution. Structural drought exacerbates these problems, as the capacity to dilute pollutants is compromised. Controlling water abstraction and limiting nutrient and pollutant inputs downstream are essential to the structural and functional recovery of biological communities and to maximizing the ecosystem services provided by the Llobregat River. [**Contrib Sci** 10:161-169 (2014)]

Introduction

In most of the world's watercourses, dramatic modifications have occurred as a consequence of their intensive use by human societies. Pollution, water abstraction, riparian simplification, bank alteration, straightening of watercourses, dam construction, and species introduction are widespread perturbations of river ecosystems. These human-driven alterations are among the global changes that in most cases do not occur independently, but mostly as combined or multiple interacting factors, so-called multiple stressors.

Natural variations may co-occur with those due to human activities, with either similar or different modes of action on ecosystems. In the Mediterranean Basin and in many arid and semi-arid areas, natural variations in climate lead to summer drought but also to extreme flooding. The natural variability of

Keywords: multiple stressors · nutrient excess · organic pollution · water scarcity · global change

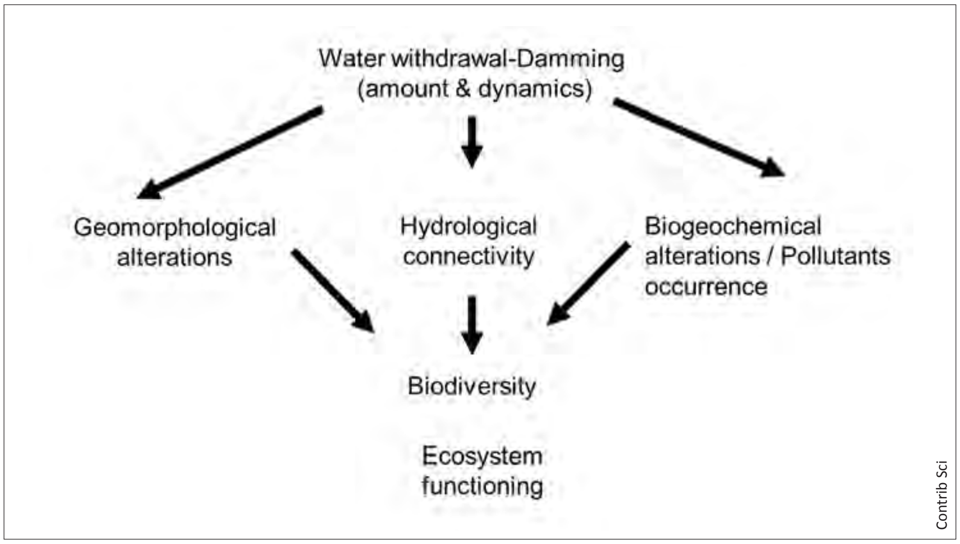

Fig. 1. Hierarchy of effects in multiple stress situations. The biological compartment and ecosystem functioning underlie all related and subsidiary stressors.

climate enhances the potential impact of water withdrawal on river ecosystems. The ratio between water demand and available water resources in the Iberian Peninsula ranges from 30% to more than 200% of the total resources in the Mediterranean basin [34]. Together, the resulting scarce water flow impedes essential ecosystem processes, therefore affecting both the structure (e.g., species richness and composition, biomass, trophic web structure) and the function of river ecosystems (e.g., productivity, use of organic matter, processing efficiency).

An alteration of natural hydrological conditions is one of the most important stressors in terms of its prevalence compared to other stressors (Fig. 1). Altering the normal hydrological pattern includes reducing the strength and frequency of flooding and of meander migration, abnormally extending the periods of hydrological stability, and lowering the incidence of post-disturbance succession [25]. Associated with hydrological alterations is the transformation of the habitat character of rivers from lotic (running waters) to lentic (standing waters) [34]. Lentification may promote higher water temperature and great evaporative losses [18], with multiple effects on the biota [40].

These examples emphasize that the occurrence of multiple stressors in a given system is not simply additive. The energy associated with disturbances is related to their intensity and frequency, which are inversely related [18,43]. Effects of disturbance depend on the associated energy and its spatial and temporal scales [39]. Moreover, hydrological modifications drive several others, or at least enhance their effects. The present paper demonstrates the relevance of hydrology through the case of the Llobregat River, a highly disturbed system in NE Spain [35]. Here, we summarize and build on several other contributions detailing the responses of the Llobregat River biota to multiple stressors [26,36].

The Llobregat River: main stressors

The Llobregat is a Mediterranean river with a strong rainfall-driven regime [24]. The river has a mean annual discharge of 14 m^3/s (Fig. 2A), although monthly averages range from <2 to 130 m^3/s. The diel water flow rate is even more variable (Fig. 2B). The hydrological year 2007-2008 was one of the driest years recently recorded, as 86% of the time the river carried less than the average water flow. During that year, flash flood episodes reaching 100–180 m^3/s occurred such that flow returned very quickly to the baseline.

The Llobregat watershed has an industrial character, particularly in its middle and lower parts. Dams and derivation channels regulate water flow. Because of these structures, large sections remain practically devoid of water during extended periods [27]. The waters of the Llobregat River are used for irrigation, industry (tannery, textile, chemical, pulp and paper), and as drinking water for the densely populated watershed. These pressures on water resources and water scarcity triggered the monitoring of the river many years ago, including some components of its biological communities. Consequently, the Llobregat is now one of the best studied rivers in the Mediterranean Basin [35].

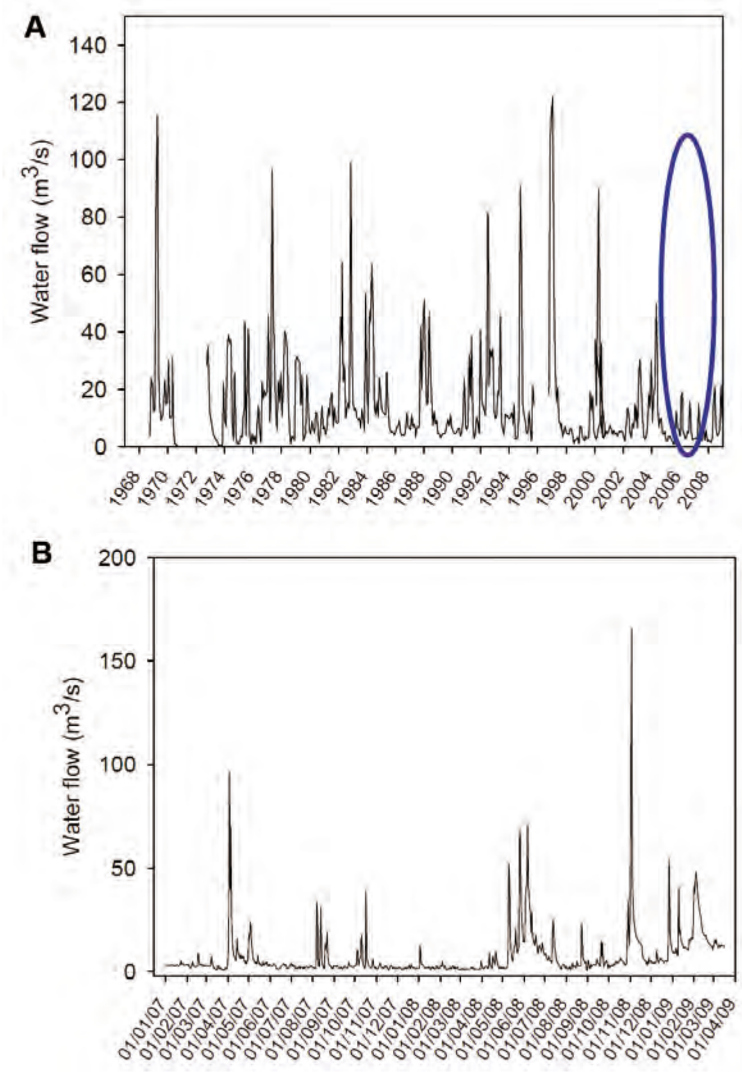

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Fig. 2. Water flow dynamics in the Llobregat River (NE Spain) close to its mouth. **(A)** Monthly average flow rates between 1968 and 2008. **(B)** Daily average flow rates in 2007–2008, during a remarkably dry period (data: Catalan Water Agency data base).

The upper-middle part of the Llobregat catchment shows evaporite-bearing geological formations, mining, and industrial activities related to potash exploitation, and therefore increasing sodium and potassium chloride concentrations in the water. In the 1990s, major infrastructural works, including wastewater treatment plants (WWTP) and a brine collector that collected and transported mining wastewater to the sea, helped to improve the ecological quality of the river, but the water quality of the middle and lower parts of the main river and some of the tributaries is still poor, due to the dense human occupation and the agglomeration of industrial facilities. Furthermore, the lower summer discharge intensifies the effects of organic matter inputs into the river coming from the WWTP.

Farming and urban settlements have caused extensive pollution of both surface and ground waters. The quality and quantity of the river and riparian habitats are poor in extended stretches. Particularly in the lower part of the Llobregat, the natural riparian vegetation has disappeared and only stands of the invasive giant reed (*Arundo donax*) are present. In the lower Llobregat, the aquifers are overexploited, and since the river dries out every summer, seawater has intruded the aquifer [8]. The water quality is poor in the lower part of the river, where pollutants such as perfluorates, pesticides, and pharmaceuticals [15,16,31] co-occur with nutrient excess, high water conductivity, and extensive habitat alteration. The effects are described below for three different situations affecting the main groups of organisms occurring in the Llobregat.

Hydrological changes and pollution effects on fish

The pollutant burden on fish from the Llobregat is well known, particularly regarding organic chemicals such as pesticides, surfactants, and plasticizers [29–32], as well as heavy metals and metalloids, which are present in substantial amounts both in the headwaters and lowlands. Severe pollution has caused frequent fish kills during the last century and even in recent years (Table 1), but these kills are probably less frequent nowadays because of the development of numerous WWTPs by the Catalan Water Agency (ACA). Organic pollutants (mainly alkylphenolic compounds) have recently been shown to have endocrine-disrupting effects on the fauna of the Llobregat, evidenced by the induction of plasma vitellogenin in male carp (*Cyprinus carpio*) and the occurrence of intersex individuals [38]. Overall, fish abundance is very low in the lowermost reaches of the Llobregat (electrofishing passes often yield no captured fish), probably due to the chronic effects of pollution.

By contrast, the effects of the massive regulation and water abstraction in the Llobregat have been largely ignored, although they are probably enormous. The Llobregat is a highly fragmented river, with a few very large dams and dozens of many small weirs for hydroelectric, irrigation, or supply purposes. This regulation is likely to affect fish populations by impeding migration and colonization, changing the flow and thermal regimes, and degrading habitats. An example of the effects of this disruption is that the eel (*Anguilla anguilla*), which has historically been present throughout most of the basin, is now largely absent in most of it and is instead confined to the delta and river mouth. Another species previously widespread in the basin and now almost gone is the chub (*Squalius laietanus*). This large cyprinid prefers pools and the water column as a microhabitat; it is thus highly likely to be affected by water regulation and abstraction and changes in its spawning grounds. The spawning grounds of many native fish such as brown trout (*Salmo trutta*), redfin barbel (*Barbus haasi*), and chub, which have lithophilic reproduction and thus require coarse substrata for spawning, have probably been altered below large dams such as the La Baells, Sant Ponç or La Llosa del Cavall. However, this aspect has not been formally studied.

Invasive species are another strong pressure on native fish assemblages in the Llobregat. As in other Iberian river basins [7], the Llobregat currently has a higher number of introduced species than native ones, with the introduced species dominating many stretches. The ecological impact of

Table 1. Fish kills recorded in the Llobregat river basin

Date	Locality	Number of dead fish and main species	Suspected cause	Ref.
November 1933	Sallent	?	Chemical pollution after drains	22
11-12 December 1957	?	?	Chemical pollution after drains resuming drought	20
January 1967	Manresa	8,000 kg	Oil spill	21
7 July 1998	Guardiola de Berguedà and Cercs	> 24,000 fish; *Salmo trutta*, *Barbus haasi*, *Cyprinus carpio*	Drought in the river due to water abstraction to a canal for hydroelectrical generation	2,10
June 1999	Sant Vicenç de Castellet	Several hundreds; *Barbus* sp.	Hydrogen peroxide and sodium carbonate	11
October 2001	Sant Boi de Llobregat	400 fish	Chemical spill	9
January 2002	Martorell	Several hundreds; *Cyprinus carpio*	Surfactant	12
April 2002	Berga	A few thousands; *Salmo trutta*	Discharge of hypolymnetic water from La Baells reservoir	42
8 May 2002	Monistrol de Montserrat	Several thousands; *Barbus* sp., *Cyprinus carpio*	Dye spill	26
November 2006	Anoia stream, between Sant Pere de Riudebitlles and Torrelavit	About 600 fish; *Barbus* sp., *Cyprinus carpio*	Chemical spill	26

these invasive species have not been studied in the Llobregat, but implications for a few species are known from other rivers in the region. For instance, the European catfish (*Silurus glanis*), introduced into the Llobregat in the last few years through the La Baells reservoir [4], has been shown to affect native fish and waterbirds in the Ter and Ebro river basins, while mosquitofish (*Gambusia holbrooki*) is known to impact the endemic, endangered cyprinodont *Aphanius iberus* [3]. The illegal stocking of exotic species is not well controlled and still frequent in Spain; it has resulted in the introduction of other, unnoticed species, such as fish parasites and zebra mussels (*Dreissena polymorpha*). In October 2011, zebra mussels were reported in the La Baells reservoir and their expansion will certainly have enormous economic impacts throughout the basin, which supplies water to the Barcelona region.

Links between environmental factors and structure and function of benthic communities: the role of priority and emerging compounds

The biological communities living in fluvial systems reflect the historical and current effects of the combined impacts of chemical, physical, and biological stressors. The effects of stressors may be additive, but synergistic interactions between natural stressors and toxicants are common phenomena in river ecosystems, mainly in industrialized countries. These interactions make it difficult to understand the effects of mixtures and establish definitive relationships between disturbances and ecosystem integrity. Over 43 million organic and inorganic compounds are commercially available, which can be released into fluvial systems and remain in sediment. Information about their occurrence and effects on organisms and ecosystems is not yet available and risk assessments have not been conducted. A subset of them (e.g., 33 compounds classified as priority substances by the Water Framework Directive) have been evaluated, classified, and finally included in the priority substance list. Estimation of the overall bulk loads of organic compounds (131 compounds) in the Llobregat show the following rank order: pharmaceuticals > alkylphenols > pesticides > illicit drugs >> estrogens [19].

Several works [5,28,33] have described the multiple toxic effects of priority and emerging compounds on benthic communities in the Llobregat. This approach is based on using multivariate techniques to assess disturbances and determine potential relationships between chemical stressors and the functional and structural composition of benthic communities. Of the 22 pesticides from seven chemical families in water and sediment, mainly triazines affect the composition of diatom biofilms [33], while conductivity and nutrients are the main factors determining macroinvertebrate distribution. Regarding functional biofilm characteristics, the percentage of variance explained by pesticides and physical and chemical variables differs among the biofilm metrics. Chlorophyll-*a* and photosynthetic efficiency are influenced mainly by the presence of pesticides, while bacterial extracellular enzymatic activities associated with the heterotrophic biofilm compartment are affected mainly by water temperature and sulfate content. Fauna and biofilm differ in their responses to pesticides, indicating that the influence of the detected compounds depends on the target organisms. Of the 20 chemicals determined, only 15% are insecticides and the rest herbicides, which are nearly always present in higher concentrations. Muñoz et al. [28] determined a potential causal association between the concentration of some pharmaceuticals and the abundance and biomass of several benthic invertebrates. Their multivariate analysis showed that the concentration of some anti-inflammatories (indomethacin and ibuprofen) and β-blockers (propanolol) correlates with higher densities and biomass of midges and worms. These results have been confirmed in laboratory conditions [23] and sediment exposure experiments [41]. Higher concentrations of pharmaceutical downstream increase the toxicological risk on communities, as was observed by Ginebreda et al. [16], who studied hazard quotient indices and their relationship with diversity indices. Areas of no risk were only observed in a few sites of the middle part of the river and in upstream tributaries.

Brix et al. [5] described that alkylphenolic compounds (APCs) played a role in the distribution of benthic communities in the Llobregat. APCs have shown a downward trend in the Llobregat over the last decade. Although the maximum allowable concentrations defined in the European Union's environmental quality standard (EQS) for nonylphenol and octylphenol have not been exceeded, there is still a potential risk of estrogenic activity. The hydrophobic metabolites of APCs may associate with organic matter in sediments and suspended particulate matter, where they interact with the lipid content of organisms, thus becoming more hazardous to the organisms in these habitats and providing a potential path for bioaccumulation and transference through the food web. Both diatom and macroinvertebrate communities show significant sensitivity to APCs that slightly change with the exposure medium, such as water vs. sediment. Other envi-

Table 2. Effects of alkylphenolic compounds (APCs) on benthic communities in the Llobregat River, expressed as percentages of partial variance explained by significant physico-chemical groups of variables. Data from multivariate redundancy analyses. Adapted from [5]

	Relative abundance of diatoms	Invertebrate density
APCs in water	6%, soluble reactive phosphorous 17%, nonylphenol di-ether carboxylate (NP2EC) and nonylphenol (NP)	17%, conductivity and soluble reactive phosphorous 9%, Nonylphenol mono-ether carboxylate (NP1EC)
APCs in sediment	8%, soluble reactive phosphorous and temperature 16%, nonylphenol mono-ether carboxylate (NP1EC)	28%, conductivity and temperature 2% sum of the nonylphenol ethoxylates

ronmental variables, such as conductivity and soluble reactive phosphorous, also contribute partly to the variance of the invertebrate density distribution along the river (Table 2).

In the studies referred to above, the identified relationships can be tentatively interpreted as cause-and-effect. In the case of pharmaceuticals, complementary experiments have confirmed several links. In other cases, the proportion of unexplained variance is high for both communities, demonstrating the potential importance of other stressors for species distribution along the pollution gradient in the Llobregat.

Community response to multiple stressors: dynamics of cyanobacterial mats and geosmin production

Geosmin is a bicyclic terpenoid by-product (4,8a-dimethyldecahydronaphthalene-4a-ol) produced by cyanobacteria and actinomycetes characteristic of wet soil and standing waters. Cyanobacteria are the main producers of geosmin in standing or slow-flowing waters, but its production only occurs under particular conditions. When present, geosmin produces an earthy-musty odor that can be perceived at very low concentrations (ca. 4 ng/l) by humans consuming the water. Its elimination requires additional water treatment before consumption and therefore significantly increases the cost of water purification. Recorded geosmin concentrations in the Llobregat have reached up to 190 ng/l (Fig. 3) and follow a remarkable seasonal pattern, with the highest values being recorded in late winter and spring.

The mass blooms of cyanobacteria coincides with peaks of the odorous metabolite geosmin in the Llobregat, suggesting that the ecological mechanisms behind its production are linked to cyanobacterial growth. Cyanobacterial toxins and odor/taste metabolites may be regulated by complex environmental factors affecting the physiological state and growth stage of the responsible species. In physiological terms, geosmin synthesis might be interpreted as a mechanism for dissipating excess carbon during growth. Synthesis of geosmin in culture has been linked to changes in cell growth caused by nutrient deficiency, as well as during the lag phase of growth, when the population is not at its optimum density.

Benthic cyanobacterial mass growth in the Llobregat starts in early January and lasts until the end of May. Benthic cyanobacteria develop largely in littoral areas or immediately downstream of dams, where waters are shallow and slow-moving (Fig. 4). A few species dominate; the filamentous cyanobacteria *Oscillatoria limosa* and *Oscillatoria tenuis* account for >90% of the abundance in the mat, while other non-cyanobacteria, such as *Vaucheria* sp. and a few diatoms, form part of the cyanobacterial mat. The occurrence of these masses is seasonal. Cyanobacterial masses progressively grow in thickness and extension, covering up to 70% of the

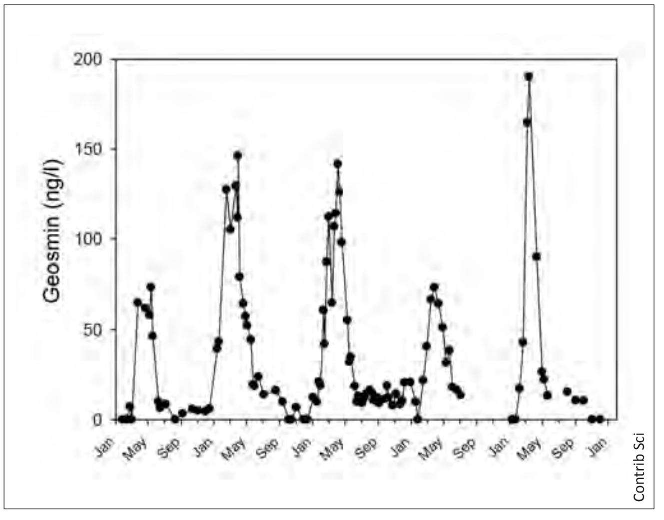

Fig. 3. Historical records of geosmin dissolved in water between 1998 and 2003. Data were obtained from Aigües Ter-Llobregat (ATLL) at the Abrera drinking water plant.

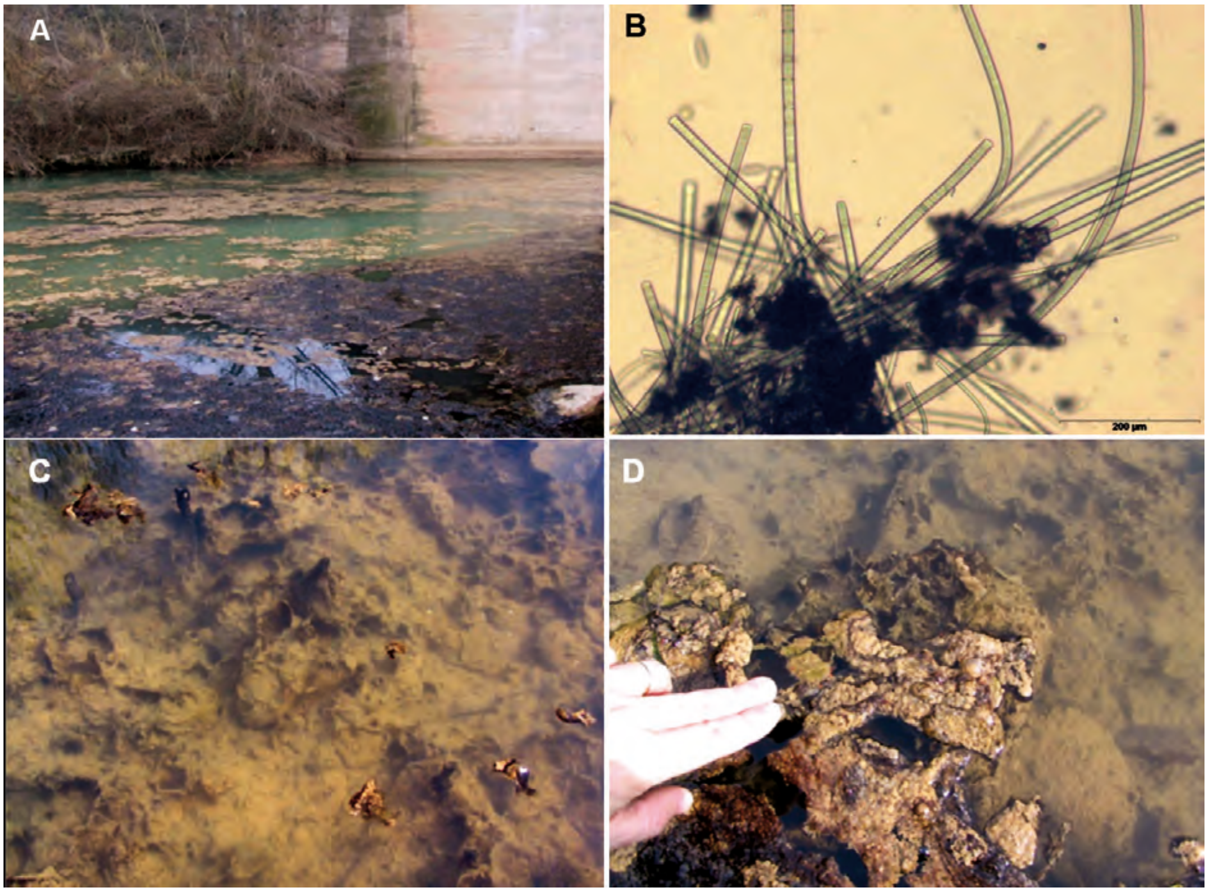

Fig. 4. Mass growth of benthic cyanobacteria in the Llobregat. **(A)** Areas of the river covered by cyanobacterial masses. **(B)** *Oscillatoria*-forming species as seen under a light microscope. **(C)** Attached masses. **(D)** Drifting masses.

total riverbed surface area. Their growth starts in shallow areas of the river (where current velocity is not much higher than 1 cm/s) and progressively extends towards the riffle zones (absent, however, in areas with fast currents). Significant fractions of the attached mats become unattached and free-floating and consequently drift downstream to colonize further areas below. Cyanobacterial mass growth terminates when the waters become warmer and flow increases (usually towards the end of May). At that time, a community of green algae dominated by *Cladophora* replaces the cyanobacterial mats. The periods when cyanobacteria prevail are characterized by high light availability and low water flow (0.25 to 0.27 m^3/s), moderate water temperature, and high phosphorus and lower dissolved inorganic nitrogen levels (lower N/P ratios) [36].

Chlorophyll-*a* concentrations in the attached and free-floating cyanobacterial mats range from 200 to 500 mg/m^2 for most of the period and correlates positively with water nutrient content. The masses have a lower geosmin concentration in the attached mats (0.55–0.97 ng/mg dry weight) than in the drifting free-floating mats (5.25–4.96 ng/mg dry weight), its concentration correlating with cell density [36]. These drifting masses have a very high density of meiofaunal organisms [14]. Chironomidae, Tardigrada, Oligochaeta, and, particularly, Nematoda account for a large number of individuals in these masses.

The conditions favoring the development of cyanobacterial masses in the Llobregat and their production of geosmin are low turbulence, full light availability, nutrient-rich conditions, and imbalanced N/P availability (low availability of nitrogen, low N/P ratios). These complete a favorable environment for the mass development of benthic cyanobacteria in the Llobregat, which achieve chlorophyll concentrations (200–500 mg/m^2) commonly recorded in eutrophic situations. The occurrence of huge cyanobacterial masses and geosmin cannot be understood without the conjoint stressors occurring in the river. Avoiding them would require a more strict control of nutrient inputs derived from diffuse and local sources, as well as hydrological restoration of the river (including removal of unused dams and the maintenance of water in the channel).

Concluding remarks

The Llobregat is a clear example of a Mediterranean river suffering from multiple stressor conditions [20,22]. It has been overexploited over the last decades, such that physical and chemical pressures constrain community diversity and species distribution along the basin [1,21]. Organism distribution is mainly determined by water scarcity, water salinity, nutrient concentration, and organic (and heavy metal) pollution. The result is a general decrease in diversity in the middle and lower parts of the main course and in the most important tributaries, where only the most tolerant species and invasive species are present. The continuous presence of weirs and reservoirs alters the distribution of the biological communities and threatens their role in river functioning. Moreover, the introduction of tolerant, non-native species adds further pressure on native species and their potential recovery. Dry conditions, common in late spring and summer, exacerbate these problems, with some reaches of the suffering low flow conditions, thereby decreasing the capacity to dilute pollution, mainly from WWTPs. Still, some reaches in the river's headwaters maintain biological communities with high diversity.

Analyses of this set of environmental indicators, grouped in biological data, ecotoxicological responses, physico-chemical characterization, and river hydromorphology, and their interactions should be part of the risk assessment procedure at Llobregat basin and site-specific scales [17]. It would provide valuable data for decision-making processed aimed at establishing management measures and programs for the more efficient monitoring of the Llobregat.

Under the scenario of rising land use changes and water scarcity in Mediterranean systems, major alterations in fluvial biodiversity compromise ecosystem integrity. In heavily managed rivers, such as the Llobregat, maintaining headwater ecosystem integrity and recovering hydrological continuity as much as possible will allow river recovery. Controlling water abstraction and limiting nutrient and pollutant inputs downstream are essential for the structural and functional recovery of biological communities and for maximizing the ecosystem services produced by the river.

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Competing interests. None declared.

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