Human Pressure and Its Effects on Water Quality and Biota in the Llobregat River

Antoni Munné, Carolina Solà, Lluís Tirapu, Carlos Barata, Maria Rieradevall, and Narcís Prat

Abstract The Llobregat River has severely been impacted by anthropogenic pressures since long time ago. The mid and lower Llobregat basin holds an important concentration of industries, agricultural activities, and urban areas, with high associated water demand and wastewater discharge. Salt mine activities, hydropower water diversion, and flow regime alteration by dams affect both the Llobregat headwaters and middle reaches. These impacts have historically caused the degradation of riparian biological communities and the loss of habitats along the river. The high amount of information available on water quality and biological community composition allows establishing a suitable monitoring program aimed to improve its ecological status. Some measures have been applied to mitigate the impacts, and Llobregat's biological quality status has progressively improved. The biological communities, mainly diatoms and macroinvertebrates, have recovered even those inhabiting the river mouth, but mostly during wet periods. However, some anthropogenic pressures still remain and Llobregat's biological status is not completely restored. The high amount of small weirs and hydropower water diversion along the Llobregat and Cardener Rivers, together with flow regime regulation by dams, riparian degradation, and point nutrient discharges (from water sewage plants) and salt debris due to mine activities, result in a poor biological quality status in the mid and lower Llobregat River. Fish fauna is the most altered community, with a high number of nonnative species present.

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The occurrence of some priority substances and emergent pollutants (e.g., endocrine disruptors, heavy metals, pesticides, flame retardants, drugs, and pharmaceuticals), even at low concentrations, further alter the biological quality. The changes in the biological community structure in the middle part of the river can be detected by using biomarkers, and these should additionally be considered as biological monitoring tools necessary for an integral ecological status diagnosis.

Keywords Biological indices, Biomarkers, Chemical status, Ecological status, Human pressure, Llobregat basin, Monitoring program, Water Framework Directive

Contents

1 Introduction

The Llobregat River is the second longest in the Catalan River Basin District (170 km long). The Llobregat basin has a total drainage area of 4,957 km^2, with an average total annual discharge of 700 Hm^3, which can range between 219 (i.e., 1944–1945) and 2,040 Hm^3 (i.e., 1971–1972) depending on year's climatology. Water flow regime also varies along the year because of the Mediterranean climate influence. High flow values are usually present in spring and autumn (between 30 and 50 m^3/s), while water flow may be scarce in summer (between 2 and 20 m^3/s). Some tributaries can even dry up in summer periods. Also, sudden floods eventually appear in spring and autumn due to heavy rain events, and water flow can rise up to 170 m^3/s (once every 2–10 years), or occasionally up to 800 m^3/s (once every 10–50 years) in the lower Llobregat River. The Llobregat ends in the Mediterranean sea close to Barcelona, where large catastrophic floods have been recorded in its vicinity (e.g., 3,080 m^3/s in 1971) [1].

The Llobregat River is heavily impacted by anthropogenic pressures as a result of its geographical location. The mid and lower Llobregat basin area holds an important concentration of industries, agricultural activities and urban areas, with an important water demand [2, 3]. River flow is regulated by three large dams in the Llobregat and Cardener headwaters which are mainly used to provide water to the lower basin (Fig. 1). Moreover, several small weirs and hydropower stations are located along Llobregat River, which, together with salt mine activities mainly located in the mid-Llobregat basin and in the headwaters of Cardener River, result in a heavy human pressure on the river ecosystem.

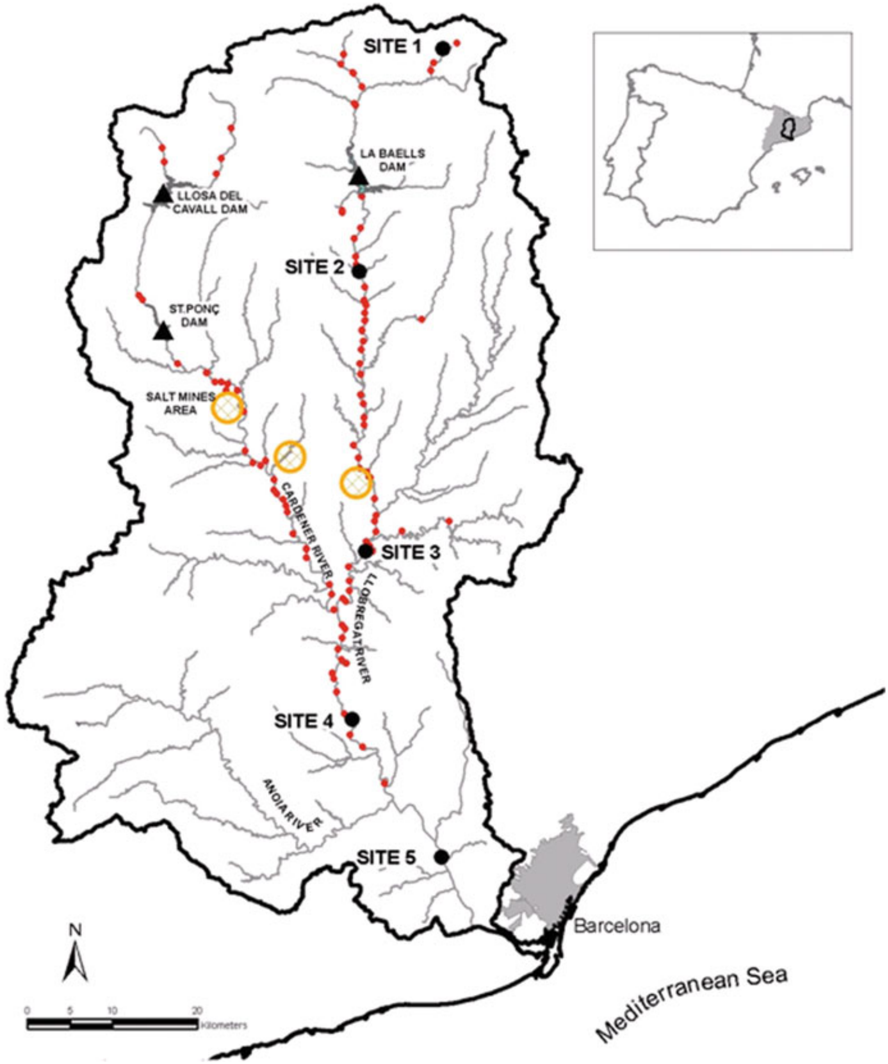

Fig. 1 The main human pressures in the Llobregat River. Major dams (*dark triangles*) and weirs (small hydropower stations highlighted using *red dots*) are located along Llobregat, Cardener, and Anoia Rivers. Also salt mine activities are shown in the basin (*orange circles*). Five sampling sites (Sites 1–5) have been selected from Llobregat headwater to the river mouth (close to Barcelona), in order to analyze quality changes along river and over time

Llobregat's water quality and ecosystem damages have been monitored for a long time by water authorities and research centers in Catalonia. The biological quality of the Llobregat River is monitored since the 1970s by using macro-invertebrate indices [4–6]. Since then, several studies and monitoring programs have been carried out to assess the river ecosystem health (e.g., [7–9]).

Furthermore, water authorities have been analyzing the chemical quality since mid-1940s. Firstly focusing on basic chemical parameters (nutrients, COD, etc.) [10], and later by analyzing priority substances, emergent pollutants, and the ecological status [11, 12]. The Water Framework Directive (2000/60/CE) introduced a new concept for water ecosystem monitoring that set apart the traditional concept of water quality, and launched a comprehensive water ecosystem analysis by using biological elements, together with hydromorphological condition analysis and chemical parameters. Macroinvertebrates, diatoms, macrophytes, and fish fauna have been more recently used to assess the biological quality in the Llobregat River and in the rest of Catalan water watersheds [13–16]. Also, priority and emergent pollutants have been monitored [17, 18], and new tools for ecosystem risk assessment have been implemented [11, 19]. Recent studies have analyzed the effect of certain emerging contaminants on biological communities in the Llobregat River [20, 21].

There is a large dataset on water quality and ecological status measurements related with the Llobregat River, and this chapter aims at analyzing the river ecological status, as well as the main effects of human pressure on the river quality. This analysis has been mostly based on five sampling sites located along the main Llobregat watercourse (Fig. 1), and the information on those sites mostly was originated from monitoring programs currently carried out (in particular those of the Catalan Water Agency and the FEM research group from the University of Barcelona). Site 1 is located upstream of La Baells reservoir in a rather natural area. Site 2 is located downstream of La Baells reservoir and is therefore submitted to flow regime alteration, but with low urban pressures. Site 3 is located in the middle part of the river, downstream of the inputs of the salt mines, and in an area with high urban discharges. Finally, Sites 4 and 5 are located in the lower Llobregat River, in the vicinity of the main industrial and urban areas, which are highly affected by sewage discharges.

2 Human Pressure and Water Management

The Llobregat River flows throughout one of the most industrialized and inhabited areas of Catalonia in its middle and lower course [22]. Waste and accidental discharges from industrial and urban activities as well as from mining activities (salt brines) are the main impacts [3, 18, 19]. High nutrient loads, salt concentrations and conductivity, and the presence of some priority and hazardous chemical substances co-occur in the lower Llobregat River [17, 18] resulting in a moderate and poor ecological and chemical status [3, 20, 23, 24]. In the same way, hydromorphological alterations by water withdrawals, flow regime alteration, and riparian occupation produce several ecosystem damages from headwaters to the river mouth.

The Llobregat River has been severely disturbed by human uses for long time ago. In the mid-nineteenth century, Llobregat River was mainly used as an energy source for the textile industry. Many weirs were built along the river, and most of them remained in place long after the mills were closed. Nowadays, most weirs still provide hydropower and they are impeding the natural flow regime and sediment transport downstream. A total of 106 hydropower stations plus 96 water withdrawal points for agricultural and urban uses are currently located along the Llobregat, Cardener, and Anoia Rivers, throughout 200 km of river watercourse (one almost every kilometer) (Fig. 1). Several weirs are placed one following the other and continuously derive a high water percentage from the river. Water is completely stored in weirs and later flowing alongside the river through canals or pipes (with an average of 1–2 km length) until the downstream hydropower station, where water is dumped back into the river. This pressure is repeated downstream again and again leaving long river reaches with poor flow or, in some cases, completely dried up. The derivation flow exceeds the natural flow regime in a total of 81 out of 106 hydropower stations (76%), which results in a scarce or null water flow in the affected river reaches.

The middle sections of the Llobregat and Cardener Rivers are also impacted by salt mine activities (Fig. 1). Saline wastes from the mining activities increase substantially the salinity of the Llobregat and Cardener Rivers. Some small tributaries like the riera Salada and the riera d'Hortons in the mid-Cardener basin, or the riera de Saldes and the Gavarresa in the mid-Llobregat are usually affected by natural salt springs resulting in high water conductivity. However, the high salt concentration along the Llobregat and Cardener Rivers appeared when salt mine industry started in Suria (1925), Cardona (1931), Salient (1932), and Balsareny areas (1954). Mine salt activities produce big saline waste dumping mountains, from the separation processes of sodium (Na) and potassium (K). Potassium is marketed, whereas sodium and other wastes have been deposited continuously near the mines and close to the river course. Saline rubbles result in a high concentration of sodium, chlorides, and bromides downstream the river due to lixiviation, in particular after rains. In natural conditions, the Cardener and Llobregat Rivers should have chloride concentrations from 40 to 100 mg/L (measured in 1915), whereas current measures are in the range of 200–500 mg/L (Sites 3–5) (Fig. 2a). Chloride concentration suddenly increases in Site 3 just downstream salt mine activities, and values remain high downstream until the river mouth, with a high variability along the year. Occasionally, high concentrations are detected in Sites 3–5, with values up to 700 mg/L of chloride. Bromide concentration increases up to 0.5–0.8 mg/L in the lower Llobregat. Hence, water conductivity usually ranges from 1,300 to 2,000 μS/cm and may rise up to 4,000 and 8,000 μS/cm. This high salt concentration affects the biological communities as well as the water uses. Moreover, water supply for urban uses needs to be properly treated to reduce chloride and bromide concentrations in order to ensure water quality, and some agricultural uses are restricted downstream.

The increasing high population inhabiting the mid and lower Llobregat basin (from 600,000 inhabitants in 1900 to 2,700,000 in 2010) mainly located close to Barcelona and nearby cities causes a high urban and industrial wastewater discharge pressure. A total of 63 sewage plants have been built by the Catalan Water

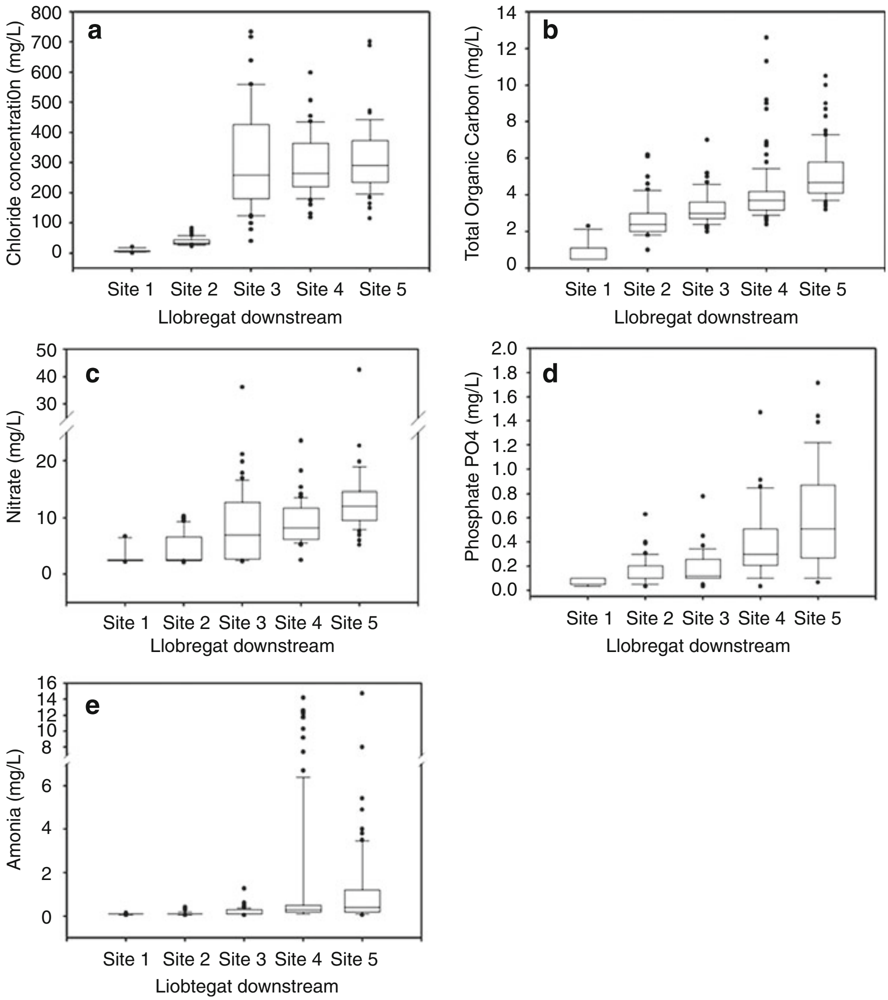

Fig. 2 Main chemical parameters analyzed along the Llobregat main watercourse (see Fig. 1). (**a**) Chloride, (**b**) total organic carbon, (**c**) nitrate, (**d**) orthophosphate, and (**e**) ammonia concentration. Values are calculated from 2007 to 2010 monthly. A total of 48 samples per site have been considered

Agency in the last 25 years in the Llobregat basin in order to mitigate wastewater effects on the river ecosystem. All sewage plants treat a total of 1,303,598 inhabitant equivalents, annually discharging up to 107 Hm^3 of treated wastewater into the river, which is equivalent to 15% of the total annual Llobregat River flow. Wastewater-treated plants discharge a total of 2,576 Mt. of N and 283 Mt. of P into the river. Therefore, a total of 44% of total nitrogen and 37% of total phosphorus are annually removed by urban and industrial sewage plans. However, the remaining nutrient load and the scarce dilution in the river cause water eutrophication. This is more evident during low flow and drought periods, when the wastewater discharge dilution is significantly reduced. Furthermore, a significant amount of treated urban wastewater (3.23 m^3/s) that comes from Barcelona urban area (1,034,297 inhabitant equivalents) is directly discharged into the Mediterranean sea through a marine outfall (2 km long).

As a result of these discharges, total organic carbon (TOC), nitrate and phosphate concentrations increase along the Llobregat River (Fig. 2b–d), and this increase is more evident from Site 2 to the river mouth (Site 5). High TOC values are mainly found in the mid-Llobregat, nearby main urban areas located close to the river (Site 3), and progressively increase downstream with values between 3 and 6 mg/L. Peak concentrations up to 10–12 mg/L are found in the lower Llobregat (Sites 4 and 5). Nutrient concentration (nitrate and phosphate), and organic matter (TOC), progressively increases from Site 2 to the river mouth (Sites 3–5) with peak values mainly found close to urban and industrial areas (Sites 4 and 5). In lower Llobregat River reaches (Sites 4 and 5), high concentrations of phosphate are also detected, from 0.3 to 0.8 mg/L, with peak concentrations up to 1.5 mg/L. Moderate nitrate values (from 5 to 15 mg/L) occur in Site 3 but quickly increase and maximum values ranging from 25 to 40 mg/L occur in Sites 3–5. On the other hand, ammonia values usually remain lower than 1 mg/L along the Llobregat River (Fig. 2e), even close to urban and industrial areas. The high impact of ammonia on river communities is due to peak concentrations nearby urban and industrial areas (Sites 4 and 5), when values are occasionally up to 15 mg/L, which may cause important detrimental effects on the biological communities [25].

Besides chemical impacts and water diversion by hydropower previously mentioned, the Llobregat River is also affected by water flow regime alteration. A significant amount of Llobregat's water withdrawal is used for urban and agricultural activities (205 Hm^3), especially in its lower water course. A large drinking water treatment plant is located close to Barcelona, which provides water from the Llobregat River to a large part of Barcelona city. To enhance the water resources of the basin, the Llobregat basin was regulated by three big dams located in Llobregat and Cardener headwaters (Fig. 1). "St Ponç" dam (24 Hm^3 of capacity), "la Llosa del Caball" dam (80 Hm^3 of capacity), both located in the upper Cardener River, and "la Baells" dam (109 Hm^3 of capacity) located at the headwaters of the Llobregat River. These dams eliminated flood events and regulated the flow along time. Flow regime attenuation can reduce habitat availability, affecting the biological community and its diversity [26], especially on fish communities [27]. Water flow stored in dams and weirs and flow regime regulation result in new habitats that can be colonized by nonnative species [28], which may have adverse impacts on Llobregat water ecosystems [29]. More than 100 nonnative species, considering riparian vegetation and aquatic flora and fauna, have been cited in the lower Llobregat River [30].

The Catalan Water Agency published the IMPRESS document (analysis of pressures and impacts on Catalan water bodies) in 2005 [22]. This document aims at highlighting and quantifying the main human pressures that could affect the good water body status according to the Water Framework Directive guidelines (http://circa.europa.eu/Public/irc/env/wfd/library?l=/framework_directive/guidance_documents/gds10srefcondspolicyssum/_EN_1.0_&a=d). Pressures from human activities were calculated and classified by combining their magnitude and possible effect on the aquatic ecosystem (sensitivity risk assessment analysis). Finally, a quality target was established for each pressure in order to quantify the risk of nonachievement of good status. The magnitude of each pressure, corrected by river sensitivity (e.g., flow regime dilution) and divided by the selected quality target, was calculated. Values near "1" (from 0.8 to 1.2) mean that the pressure corrected by the river sensitivity is close to the quality standard (low risk), values over "1" (between 1.2 and 2) mean that the pressure exceeds the quality threshold (quality target) and the risk is moderate, and values over "2" mean high risk. Besides, values under 0.8 were classified as without risk. Risk assessment was calculated for a total of ten human pressures identified in the Llobregat River (Table 1). Results showed that the main highlighted pressures were due to urban and industrial wastewater discharges into the river. A total of 29 out of 80 water bodies identified in the Llobregat basin (34%) are affected by wastewater discharges. Also, water diversion for hydropower affects a total of 16 water bodies (19%), salt mine wastes affect a total of 13 water bodies (15%), flow alteration by upstream dam regulation affect 10 water bodies (12%), and river channelization affect 10 water bodies (12%) mainly located downstream. Invasive species also produce a high pressure on the Llobregat River water bodies, affecting a total of 18 water bodies (21%),

Table 1 Main human pressures identified in the Llobregat basin. Number and percentage of affected water bodies have been classified according to the magnitude of pressures and risk assessment. River water bodies in the Llobregat basin have an average length of 15 km

Human pressures on Llobregat River	High risk		Moderate risk		Low risk		With some risk	
	No. of water bodies	%	No. of water bodies	%	No. of water bodies	%	No. of water bodies	%
Hydromorphological pressures								
Connectivity lost (weirs and dams)	3	3.6	3	3.6	2	2.4	8	9.5
River canalization	4	4.8	3	3.6	3	3.6	10	11.9
Water withdrawals	1	1.2	0	0.0	2	2.4	3	3.6
Flow regime alteration (dam regulation)	3	3.6	3	3.6	4	4.8	10	11.9
Water diversion by hydropower stations (flow reduction)	14	16.7	0	0.0	2	2.4	16	19.0
Chemical pressures								
Urban and industrial wastewater discharge	15	17.9	10	11.9	4	4.8	29	34.5
Rainwater sewage system discharges	5	6.0	0	0.0	0	0.0	5	6.0
Salt mine activities	3	3.6	6	7.1	4	4.8	13	15.5
Other pressures								
Invasive species	3	3.6	7	8.3	8	9.5	18	21.4
Accumulated pressure	29	34.5	16	19.0	6	7.1	51	60.7

although the main pressures were classified to have low and moderate levels of impacts. These pressures have continuously increased in the last years. The two main high pressures identified in the Llobregat basin were urban and industrial wastewater discharge, and water diversion by hydropower uses, mainly classified as pressures producing high risk. A total of 15 water bodies for urban and industrial discharge (18% of the total river water bodies) and 14 water bodies for water diversion by hydropower (17% of the total river water bodies) were classified as having a high risk (Table 1). Other detected pressures were poor river connectivity that affected 8 river water bodies (9%), rainwater discharges affecting a total of 5 water bodies (6%), and water withdrawals mainly due to agricultural activities and water supply to urban areas, affecting a total of 3 water bodies (4%). Pressures and their magnitude progressively increase downstream. Thus, water bodies located in the mid and lower Llobregat River show higher risk due to several pressures (water withdrawal, urban and industrial wastewater discharge, water diversion, flow regime alteration, invasive species, salt mine waste, etc.). However, the headwaters are mainly affected by hydropower water diversion and low connectivity because of the presence of high amount of weirs. A total of 51 out of 80 river water bodies (61%) have some risk to nonachieve a good ecologically status due to human pressures on the Llobregat basin.

3 Biological Communities and Quality Assessment

The Water Framework Directive (2000/60/EC) (WFD) and the Priority Substances Directive (105/2008/EC) provided significant changes for water quality assessment in the European aquatic ecosystems [31–33]. The application of these Directives requires using a new monitoring program, which EU Member States were bound to apply since 2007, according to the WFD requirements. Both, ecological status and priority substances must be taken into account in order to establish a comprehensive water status diagnosis. Chemical and biological elements must be combined to set the final water quality status. Ecological status assessed using biological elements, together with the chemical status, have been implemented in the Llobregat basin by the Catalan Water Agency since the new Monitoring Program started in 2007 [24]. Moreover, some Catalan research centers have also been analyzing the chemical and ecological status through research projects launched several years ago (e.g., ECOBILL, ModelKey, and KeyBioeffects projects).

In Europe, diverse biological indices and metrics have been developed for ecological status assessment in rivers using macroinvertebrate [34, 35], diatoms [36], macrophytes [37], and fish communities (e.g., [38]). Biological indices have also been applied in Catalan Rivers since long time ago, basically developed in research centers [3, 7, 9, 14], and more recently applied by Water Authorities following the WFD requirements [13, 16, 24, 39]. The assessment of biological quality in rivers has been developed and enhanced by contributions of several research centers and water authorities in order to achieve the normative definitions of WFD and their compliance with a gradient stressor.

3.1 Historical Perspective on Biomonitoring

The study of freshwater communities in the Llobregat River and their use as bioindicators has a long tradition [3]. Ramon Margalef (University of Barcelona) in 1951 made a first attempt to use algae as indicators in his monograph entitled "Indicator organism in Limnology" [40]. Moreover, Margalef adapted the Saprobic system from central European countries to Spanish rivers [41]. Much of this work was done in the Llobregat basin, and in 1978, he helped to start a survey of the rivers Llobregat and Besos, which was the seed for the future monitoring programs currently being carried out. A first biotic index using macroinvertebrates called "BILL" was later defined in the Llobregat and Besos Rivers [4]. Data were presented in a series of papers [5, 9, 42]. A regular biomonitoring program for the Llobregat basin was definitively set up in 1994 after a series of large floods by the FEM research group (University of Barcelona). Since then, a Llobregat monitoring program has been established and continued mainly using macroinvertebrate communities and chemical parameters [7, 8]. The sampling site network carried out by the ECOBILL monitoring program consists of 25 sampling sites covering the main stream and permanent and temporary tributaries in the Llobregat basin. The IBMWP index uses macroinvertebrates at family level, which requires a multihabitat sampling and an extensive searching of macroinvertebrate families (GUADALMED protocol: [43]). Each sampling site is visited twice a year (spring and summer), and data are available on http://ecobill.diba.cat.

The evolution of biological water quality by using macroinvertebrate communities (IBMWP index) in 25 sites of the basin has been analyzed in the Llobregat River through the FEM research group data (Fig. 3). The IBMWP index is a useful quality index based on macroinvertebrate community composition at the family level [44], which has been commonly used by Spanish Water Authorities. The percentage of sampling sites classified in each of the five quality classes can be compared from 1997 to 2010 (Fig. 3a). From 1997 until 2006, close to 50% of sites were classified below a good quality level with some changes along years and high variability between dry and wet years. The worst quality values coincide with dry years (e.g., 2004, 2006, and 2008), when close to 60–70% of sites were classified below good quality status. The importance of dry years in the biological quality assessment of Mediterranean rivers was studied by Munné and Prat [39], who concluded that reference condition values should be lowered for dry periods in order to properly interpret quality status and compare it along time. In wetter years the biological quality of Llobregat's water improves, also because of the urban and industrial sewage treatments implemented in its basin.

Furthermore, when the IBMWP evolution is analyzed over 17 years (from 1994 to 2010) in the five sites considered in this study (Fig. 3b) is evidenced that the

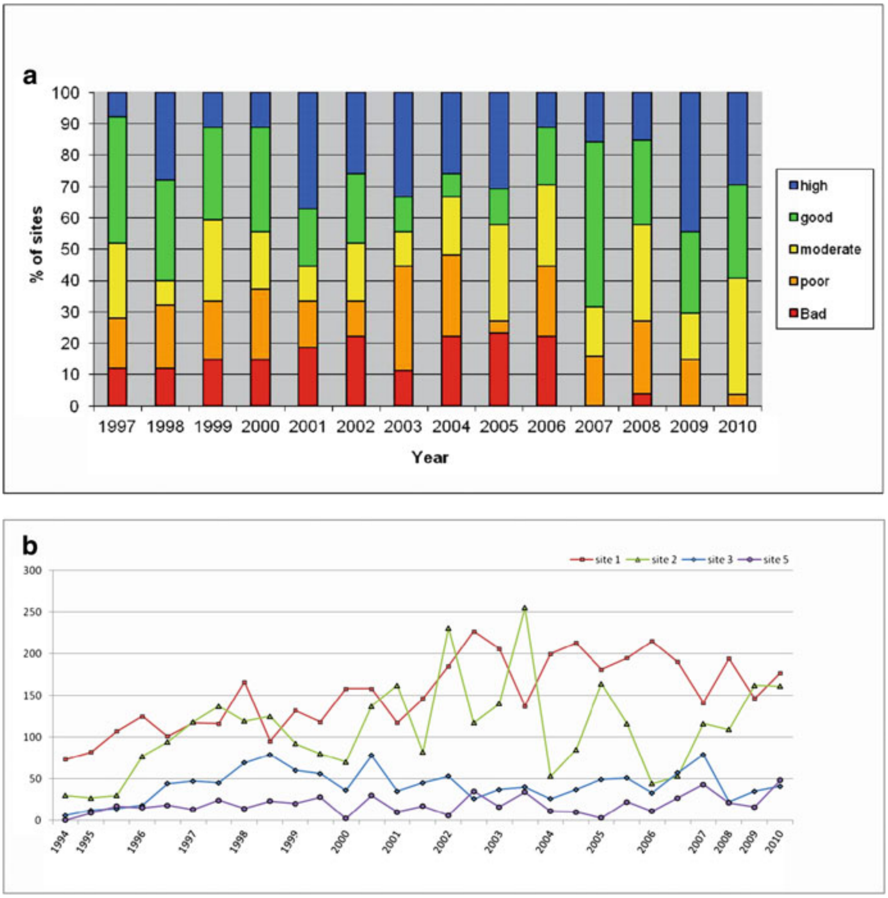

Fig. 3 Temporal evolution of the biological quality in the Llobregat basin using the IBMWP index. (**a**) The percentage of sampling sites classified in five quality classes is shown from 1997 to 2010. A total of 25 sampling sites covering the main Llobregat watercourses and tributaries have been selected from the ECOBILL database (FEM research group, University of Barcelona). (**b**) Changes along time (from 1994 to 2010) of the IBMWP index are shown for selected sites located along the Llobregat River from headwaters to the river mouth (see Fig. 1)

water quality of the upper part of the basin has continuously been improved (Sites 1 and 2), while the lower part (Sites 4 and 5) is still remaining in a poor or moderate quality status. All sites show low IBMWP values at the beginning of the analyzed period due to the large floods occurred in 1994. High floods dragged most macroinvertebrate communities, and this event provided a good basis to analyze the capacity of macroinvertebrate colonization and biological quality improvement along the Llobregat River. While quality values continuously increased along time in headwaters (Sites 1 and 2), with some interannual fluctuations, water quality values did not recover so much along time in downstream locations (Sites 3 and 5).

In the mid-Llobregat part (Site 3), values never achieved the good status (IBMWP values upper to 60), probably due to the influence of salt mines and local urban inputs. In Site 2 values increase as in Site 1, but largely fluctuate from year to year with some very low values during dry periods (e.g., 2004 and 2006). In 2002 the Llobregat headwaters located before salt mine activities and main urban discharges completely restored the high biological quality, whereas Llobregat stretches below main human pressures remained in a lower quality class, only reaching moderate status. The evolution of the lower Llobregat water quality and its relationship with the hydrology has been recently studied [45]. Comparison of these data with the old data from 1979 and 1980 is not possible due to the different sampling methods. However, values of the biological index BILL indicate even a worst situation in the river at that time, especially in the middle and lower parts [5, 46].

3.2 *Current Ecological and Chemical Status*

Suitable Mediterranean river type-specific indices for each biological quality element (BQE), required by the WFD (macroinvertebrates, diatoms, macrophytes, and fish), have been applied for the biological quality assessment in the Llobregat basin (Table 2 and Fig. 4). Quality classes must be later combined using BQE values according to "one out, all out" criteria [47] in order to establish the final biological quality class (Table 2 and Fig. 5a). This is a restrictive procedure since the worst biological quality item is used to set the final biological quality status.

Biological data were obtained from spring samples (from April to June) by the Catalan Water Agency through its Monitoring Program (2007–2012). The data available up to now were obtained during 2007–2010. Macroinvertebrates and

Table 2 Number and percentage (in parentheses) of river water bodies classified in five biological quality classes for each biological quality element (macroinvertebrates, diatoms, macrophytes, and fish fauna) in the Llobregat basin. Chemical status is also shown using two quality classes according to the Water Framework Directive. Data are provided by the Catalan Water Agency monitoring program carried out from 2007 to 2010. A total of 80 river water bodies have been established in the Llobregat basin

Biological elements	Quality classes					Water bodies without data
	High	Good	Moderate	Poor	Bad	
Macroinvertebrates (IBMWP index)	41 (51%)	16 (20%)	11 (14%)	7 (9%)	0 (0%)	5 (6%)
Diatoms (IPS index)	24 (30%)	17 (21%)	3 (4%)	16 (20%)	8 (10%)	12 (15%)
Macrophytes (IBMR index)	0 (0%)	2 (3%)	1 (1%)	5 (6%)	2 (3%)	70 (87%)
Fish (IBICAT index)	5 (6%)	3 (4%)	20 (25%)	3 (4%)	18 (22%)	31 (39%)
Biological quality	9 (11%)	14 (18%)	6 (7%)	33 (41%)	15 (19%)	3 (4%)
	Good		Bad			Water bodies without data
Chemical status	66 (83%)		10 (12%)			4 (5%)

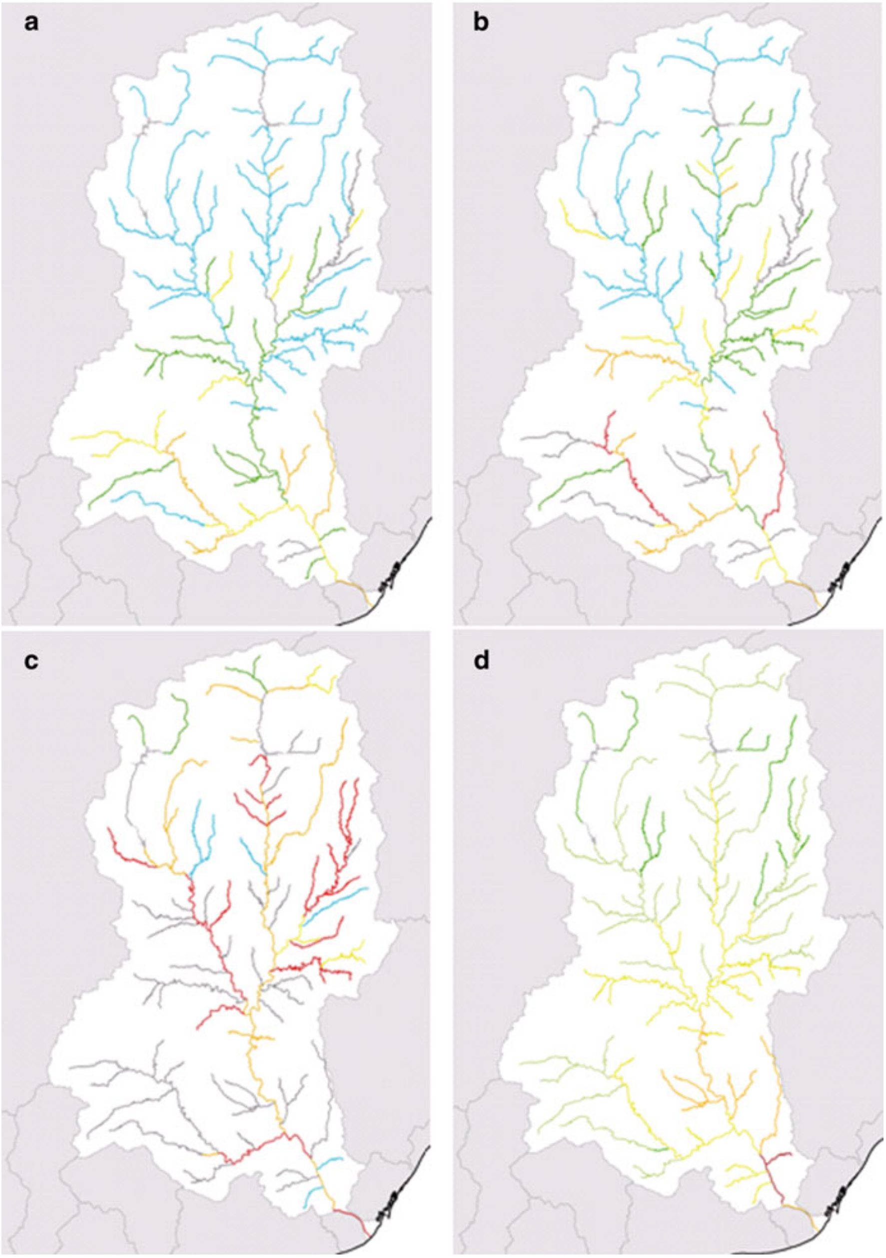

Fig. 4 Biological quality in the Llobregat basin (2007–2010). High quality class is shown in *blue*, good quality in *green*, moderate quality in *yellow*, poor quality in *orange*, and bad quality in *red color*. Water bodies without data are shown in *gray*. Water quality is measured by using: (**a**) macroinvertebrates (IBMWP index), (**b**) diatoms (IPS index), and (**c**) fish (IBICAT index). (**d**) The number of total nonnative aquatic species is shown for each river water body. Water bodies with a total nonnative species from 1 to 30 (considering riparian vegetation and all aquatic organisms) are shown in *dark green color*, from 30 to 60 with *light green*, from 60 to 80 with *yellow*, from 80 to 100 with *orange*, and more than 100 are shown in *red color*

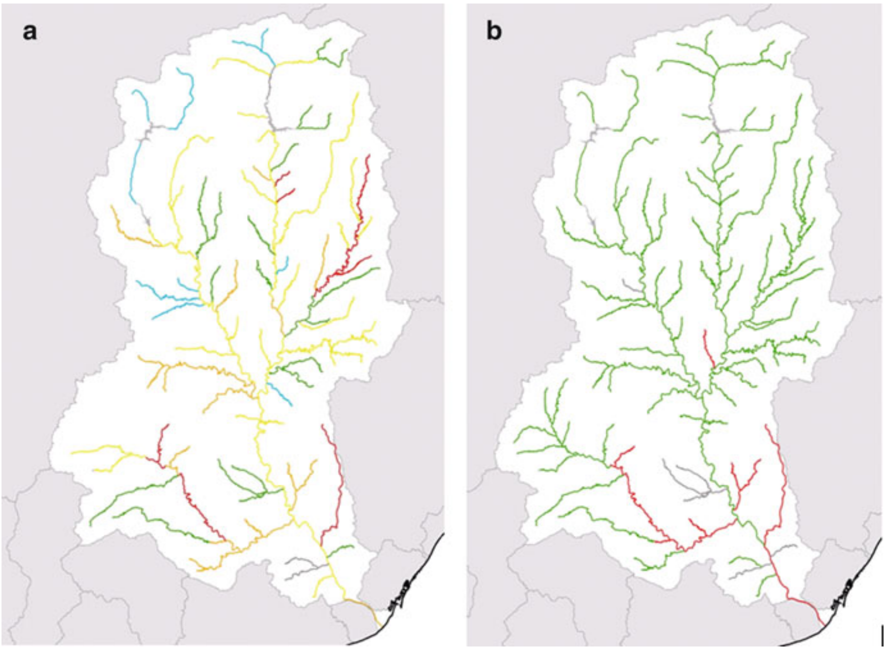

Fig. 5 Biological quality and chemical status in the Llobregat basin (2007–2010). (**a**) High ecological status is shown in *blue*, good quality in *green*, moderate quality in *yellow*, poor quality in *orange*, and bad quality in *red color*. Water bodies without data are shown in *gray*. (**b**) Good chemical status is shown in *green* and bad quality in *red color*

diatoms were sampled annually for river water body at risk, and at least twice along the monitoring period (2007–2012) in the remaining water bodies without relevant human pressures. Macrophytes and fish were sampled at least once from 2007 to 2012 in all water bodies. The IBMWP for macroinvertebrate [44], the IPS for diatoms [36], the IBMR for macrophytes [48], and the IBICAT index for fish fauna [15] have been used to establish the biological quality in the Llobregat basin by the Catalan Water Agency. Samples were gathered following specific sampling protocols, and data are available in the Catalan Water Agency web page. These indices mainly came from European projects (e.g., [38, 49]), and some of them have commonly been applied in Spain for long time (mainly IBMWP and IPS indices).

A total of 57 water bodies in the Llobregat basin (71%) achieve high or good quality status according to the IBMWP index based on macroinvertebrate communities (Table 2, Fig. 4a). Similar results but slightly worse are found by using diatoms as a BQE (Table 2, Fig. 4b). Quality objectives (good and high quality) are achieved in 51% of river water bodies (41 out of 80 water bodies established in the Llobregat basin) when diatom index is used (IPS index). Diatoms are very sensitive organisms for organic pollution, and they have a rapid response to chemical disturbances [36]. Low-quality values are found in more river water bodies than using macroinvertebrates specially in water bodies classified as high quality with macroinvertebrate index only obtain a good status with diatom indexes. Macroinvertebrates are mainly sensitive to organic pollution, but they can have a slower and sustained response to eutrophication than diatoms [50]. However, in most cases the IBMWP index values were quite consistent with diatoms results, which are low in mid-Anoia and lower Llobregat Rivers. Low IPS indexes are found near urban and industrial areas receiving high nutrient loads, and mainly in the Anoia, low Cardener, and mid and low Llobregat watercourses. A total of 23% river water bodies for macroinvertebrates (18 out of 80 water bodies), and 34% for diatoms (27 out of 80 water bodies), do not achieve good biological quality in the Llobregat basin. Moreover, 6% of river water bodies for macroinvertebrates (5 out of 80 water bodies), and 15% for diatoms (12 out of 80 water bodies), have not been analyzed yet. These water bodies will be further sampled along the monitoring program period (2007–2012).

Macrophytes have scarcely been used in the Llobregat River for quality purposes so far. Quality indices based on macrophytes are currently tested and still under discussion [51]. Several macrophyte quality indices are currently available (IM, IVAM, etc.), and some of them have been applied in the Llobregat basin [52]. More recently the IBMR index [48], which was intercalibrated for the Mediterranean rivers according to the WFD requirements, has been applied by the Catalan Water Agency in the Catalan Rivers, but only data of ten river water bodies are nowadays available in the Llobregat basin (Table 2). From these ten sampled river reaches, a few show good quality and the majority (eight out of ten) are classified in a moderate, poor, and bad quality status. Macrophyte-based indexes are heavily dependent on the substrata type and on the hydro-morphological conditions. Also inappropriate sampling protocol can negatively affect the output. Results should be therefore carefully considered because of insufficient knowledge on its application in Mediterranean rivers. Macrophyte indices, and its interpretation in terms of water quality, need to be improved to be properly used for ecological status assessment in the Llobregat River.

Fish fauna shows the worst quality scenario from any of the measured BQEs. Only 10% of water bodies (8 out of 80 water bodies) show fish communities dominated by native species (Table 2, Fig. 4c). Fish fauna are sensitive not only to water pollution, but also to morphological and flow alterations, river discontinuity, and habitat loss [27, 53]. Low-quality values using fish fauna denote lack of suitable habitat conditions, and hence hydrological alteration and water abstractions can considerably affect native fish community composition. Moreover, additional threats such as exotic alien species invasions, basically introduced for fishing, negatively affect fish communities and prevent them to achieve a good biological quality. The main Llobregat watercourse, and mainly its lower part, hosts a high number of nonnative fish species (Table 3, Fig. 4d). Close to 100% of fish species in the mid and lower Llobregat main watercourse are nonnative. A total of three to five nonnative fish species are usually present in the mid and lower Llobregat. Also, over 100 nonnative species (considering all riparian and aquatic flora and fauna) have sometime been cited in the lower Llobregat River [30]. Nonnative species are

Table 3 Quality indices and main metrics of the biological quality elements along the main Llobregat watercourse (see Fig. 1) using the biological quality elements (macroinvertebrates, diatoms, macrophytes, and fish fauna) required by the Water Framework Directive to assess the ecological status in rivers

Biological quality elements	Quality indexes or biological metrics	Site 1	Site 2	Site 3	Site 4	Site 5
Macroinvertebrates	IBMWP	155 (high)	138 (high)	82 (good)	70 (good)	44 (moderate)
	EPT	16	11	6	4	3
	S (num. fam.)	34	31	28	21	14
Diatoms	IPS	19.6 (high)	15.3 (high)	11.8 (moderate)	11.7 (high)	6.7 (moderate)
	TDI	28.8	89.8	90.8	81.1	96.2
	H′	1.45	2.52	3.94	4.15	3.51
	S (num. sp.)	15	24	34	38	34
Macrophytes	IBMR	13 (good)	3 (bad)	nd	9.25 (poor)	nd
	S (num. sp.)	18	3	nd	19	nd
Fish	IBICAT2010[a]	4.0 (moderate)	7.5 (poor)	3.7 (bad)	9.9 (poor)	8.4 (poor)
	S (num. sp.)	3	3	5	5	4
	S (num. alien sp.)	1	1	5	5	3
Alien sp. (all aquatic communities)[b]	S (num. sp.)	37	66	78	97	141

[a]Results for each site are calculated according to its reference condition and type-specific metrics. That is the reason why values are not comparable. Quality classes can be compared between sites

[b]The total amount of alien species has been calculated trough EXOAQUA project (supported by the Catalan Water Agency and carried out by the CREAF). All nonnative aquatic species cited in several studies and projects were collected in a large database (EXOAQUA). Riparian vegetation, vertebrate and invertebrate fauna, and also algae and macrophytes were considered

basically distributed in the lower basin, close to the river mouth and near urban areas. Headwaters near nonimpacted areas held communities with less than 30 aquatic nonnative species. Nonnative species invasions are considered a high risk for aquatic ecosystems due to their usually high dispersal capacity [54]. A database with nonnative and invader species cited in the Catalan aquatic ecosystems can be consulted online through the Catalan Water Agency web page (http://aca-web.gencat.cat).

Finally, high and good biological quality, combining macroinvertebrate, diatoms, and fish biological elements, are achieved in 29% of the Llobregat water bodies (23 out of 80 water bodies) (Table 2, Fig. 5a). Biological quality has been set out using the worst three BQEs measured (macroinvertebrate, diatoms, and fish fauna). Due to scarcity of current data, macrophytes are not considered yet. Results show high and good quality in headwaters, basically rivers upstream the major human pressures (salt mine activities and urban discharges), and small streams far from urban and agricultural areas, mostly tributaries. The middle and lower Llobregat River, in areas below salt mine and nearby urban and industrial areas, show many water bodies with moderate, poor, and bad biological quality. The main Llobregat watercourse below the dam shows a moderate biological quality mainly due to fish community alterations, since macroinvertebrate and diatoms provide a good quality. The worst biological quality is at the lowest Llobregat main watercourse, close to Barcelona, and in some tributaries flowing throughout high-industrialized areas, like as the Anoia and Rubi Rivers, both located in the lower Llobregat basin.

By analyzing the evolution of biological patterns and quality indices along the Llobregat main watercourse (Table 3), we can observe how the number of macroinvertebrate and diatom taxa decreases from the Llobregat headwater (Site 1) to the river mouth (Site 5). From a total of 34 families of macroinvertebrates that are found in the Llobregat headwaters, only 14 families are sampled at the lower part. Also, the number of families of Ephemeroptera, Plecoptera, and Trichoptera (EPT) diminish throughout the main Llobregat watercourse, from 16 families sampled in headwater to only three families close to the river mouth (Baetidae, Caenidae, and Hydropsychidae). Besides, a clear change of macroinvertebrate taxonomic composition along river is detected. Some taxa progressively disappear downstream, while other ones appear. Macroinvertebrate families that disappear are more sensitive to water pollution, and prefer well oxygenated and cold water. Some families of Plecoptera, Trichoptera, Ephemeroptera, and Diptera are commonly found in headwaters, over the salt mine activity and main water flow alteration by dams (Site 1) (e.g., Chloroperlidae, Perlidae, Perlodidae, Nemouridae, Leuctridae, Ephemerellidae, Leptophlebiidae, Rhyacophilidae, Limnephilidae, Athericidae, and Glossossomatidae), but some of them disappear downstream of dams (Site 2) (e.g., Chloroperlidae, Perlidae, Perlodidae, Nemouridae, Athericidae, and Glossossomatidae). Also, downstream salt mine activities (Site 3) more tolerant families are found (e.g., Baetidae, Hydropsychidae, and Simulidae), and major taxonomic groups disappear (e.g., Leuctridae, Ephemerellidae, Leptophlebiidae, and Rhyacophilidae). On the other hand, some other taxa, mainly Trichoptera

(Brachycentridae and Leptoceridae), are not found in the Llobregat headwaters (Site 1), but they are often sampled in the mid-Llobregat, even downstream of salt mine areas (Sites 2 and 3). Those taxa are also sensitive to water pollution and human pressures and, therefore, they indicate good biological quality. Sites 1 and 2, respectively, located upstream and downstream of the flow regulation caused by La Baells dam show high-quality status by using the macroinvertebrate index. Evenly Site 3, located downstream major salt mine activities and small urban discharges, shows a good quality status by using macroinvertebrates (IBMWP). On the other hand, only few macroinvertebrate taxa inhabit the lower Llobregat River, heavily affected by urban and industrial discharges (Sites 4 and 5). In the lower Llobregat River, the macroinvertebrate fauna is basically composed by Ephemeroptera (Baetidae, Caenidae), Trichoptera (Hydropsychidae), Diptera (Chironomidae), some Mollusca (Physidae), and Oligochaeta, taxa resistant to pollution. Nevertheless, the richness of macroinvertebrates is very low nearby the river mouth (Site 5), and biological quality by using macroinvertebrates is at most moderate (just under the quality target). In summary, macroinvertebrate community alteration slightly change under salt mine activities, and most quality damages are detected downstream urban and industrial discharges (Sites 4 and 5), where biological indices are not achieving the quality objectives by using macroinvertebrate community (IBMWP index).

The number of taxa (species) increases from headwaters (15 species sampled in Site 1) to the river mouth (34 species found in Site 5). Most of the diatoms species inhabiting in the lower Llobregat River are highly tolerant to water pollution and nutrient loads (e.g., *Cocconeis placentula* and *Nitzschia inconspicua*), whereas less tolerant species are found upstream (Sites 1 and 2) (e.g., *Achnanthidium biasolettianum*). Water quality measured by using diatoms (IPS) and macroinvertebrates (IBMWP) is quite similar. Quality values decrease downstream, although major changes are downstream of salt mine activities, where quality class shifts from good to moderate. Also, trophic index (TDI) increases downstream, from 28.8 in Site 1 to 96.2 in Site 5 (Table 3), which coincides with an increasing nutrient load. The fish community is the most restrictive BQE applied in the Llobregat River. Quality values using the IBICAT index only show near good quality status upstream dams (Site 1), where the fish quality class is moderate, and fish communities are dominated by native species (e.g., *Salmo trutta*, *Barbus haasi*). In the mid and lower Llobregat main watercourse, below dam flow regime regulation, and nearby the high urban areas, quality status is poor or bad due to the poor hydromorphological quality, habitat loss, riparian degradation, and high abundance of nonnative fish species found (e.g., *Lepomis gibbosus*, *Cyprinus carpio*, *Alburnus alburnus*, and *Phoxinus* sp.).

Regarding the chemical status, a total of 97 priority substances and group of substances (isomers, metabolites, etc.) have been analyzed in the Llobregat basin according to the 105/2008/EC Directive. Atomic fluorescence spectroscopy for mercury, inductively coupled plasma mass spectrometry for heavy metals, headspace extraction procedure for solvent substances, solvent extraction with simultaneous derivatization for pentachlorophenol, and solid-phase stirred bar extraction for the rest of organic compounds [55–57] are used in order to analyze priority substances and to set out the chemical status. All chemicals were also analyzed or confirmed using GC–MS according to the 2009/90/EC Directive. From these 97 substances, 42 are included in the Annex I of the 105/2008/EC Directive, while 55 remaining substances are required by Spanish-national laws, or are likely to be found in Catalan Rivers due to industrial or agricultural activities. Only substances with thresholds provided by the 105/2008 Directive (EQSs) were used for chemical status assessment. Values of heavy metals (lead, cadmium, mercury, and nickel), chlorinated solvents, pesticides (chlorine, phosphorus, and triazine), polycyclic aromatic hydrocarbons, and endocrine disruptors (nonylphenols, octilphenols, and brominated diphenyl ether compounds) are analyzed according to the EQS provided by the 105/2008/EC Directive.

The good chemical status is achieved in 83% of river water bodies (66 out of 80 water bodies) in the Llobregat basin (Table 2, Fig. 5b). A total of ten river water bodies do not achieve good chemical status. Unfulfilled environmental quality standards (EQS) are located close to industrial areas, in the lower part of Llobregat, Rubi, and Anoia Rivers. Also, additional unfulfilled quality standards exist in some small streams located in the mid-Llobregat basin, close to the Manresa city industrial area (riu d'Or). Pesticides and endocrine disruptors are the main substances responsible of quality standard failures. Endocrine disruptors are mainly found in the lower main Llobregat watercourse. A total of nine river water bodies do not achieve the quality standards in the Llobregat basin, from which four are located in the mid and lower Llobregat below Cardener River. The remaining high endocrine disruptor concentrations are found in the lower Anoia River, the Rubí stream, and riu d'Or, all located downstream of big industrialized zones. Nonylphenols (EQS: 0.3 μg/L) and octilphenols (EQS: 0.1 μg/L) are present with an average concentration of 0.4–0.8 μg/L. Similar endocrine disruptors concentrations have been found by other authors close to industrial areas, where similar compounds are mainly used in industrial processes [58]. Moreover, chlorinated pesticides, triazines, organophosphates, and some miscellaneous compounds are also found in the lower Llobregat basin close to industrial and agricultural areas. Pesticides do not achieve quality standards in five water bodies (6% of the total Llobregat River water bodies), and they are mainly located in the mid and lower Llobregat, the lower Anoia River, riu d'Or, and Rubí stream. Mostly trifluralin and also hexachlorocyclohexanes (lindane) are the main hazardous substances found over their EQS. Trifluralin is found in tributaries of lower Llobregat (Rubi stream) with an average concentration of 0.05 μg/L (slightly over its EQS value: 0.03 μg/L). Lindane is found in riu d'Or, in the mid-Llobregat River with an average value of 0.03 μg/L (EQS: 0.02 μg/L). Regarding triazines, organophosphates, and miscellaneous compounds, they do not achieve quality standards in two river water bodies, basically located in the lower Anoia River, and in Rubí stream, nearby industrial areas. Chlorpyrifos and chlorfenvinfos are the most detected compounds, though mainly found at low concentrations. Measured chlorpyrifos show an average concentration of 0.03–0.09 μg/L (EQS: 0.03 μg/L), whereas chlorfenvinfos are detected at 0.1–0.15 μg/L (EQS: 0.1 μg/L). Most pesticides have been detected slightly over the EQS values and their threshold detection. Therefore, they must be tentatively considered and later evaluated along time to be confirmed. Heavy metals are also found near industrialized areas in the lower Llobregat and Anoia Rivers. Nickel values are detected over the quality standard (EQS: 20 μg/L) in three water bodies (in lower Anoia and Llobregat Rivers), with an average value of 30 μg/L.

4 New Tools for Ecosystem Risk Assessment

Community-based monitoring approaches such as those described above, provide useful information on composition and structure, but do not provide clear insight into the impact of pressures of ecosystem functioning neither on specific pollution effects on river biota. Community-based indexes can only detect relatively strong effects that usually involve the eradication of one or several species from a particular site. Thus, they cannot diagnose low levels of ecological impairment caused by sublethal physiological effects. In relation to this, the development of innovative ecological assessment methods such as *i*n *s*itu *b*ioassays (ISBs), that employ caged species deployed at sites of concern, offer great potential for use in a tiered assessment scheme since they show good abilities to discriminate among chemical pressures [59]. The development of biomarkers in macroinvertebrate species has allowed identifying major pollutants with detrimental effects on river biota [23, 59]. ISBs per se can provide valuable information on functional processes such as changes in food processing and hence on trophic food webs. For example, the use of postexposure feeding inhibition responses of *Daphnia magna* transplanted across different locations along the Llobregat River basin has allowed characterizing "hot" sites whose water pollutants impair grazing rates [59]. Such detrimental effects may resolve in the elimination of filter feeders like tricopters. The use of a large set of biomarkers that involve several metabolic and detoxication paths may also be useful to indentify particular pollutants that are affecting river biota. Biomarkers can be measured in field collected organisms and then may inform us about the physiological state of organisms inhabiting those sites [19, 23]. If biomarkers are combined with the analysis of pollutants, it is possible to correlate specific effects with putative stressors [19, 21]. Nevertheless, one of the greatest advantages of the above-mentioned tools is its combination. When ISBs, biomarkers, and chemical analyses are combined and related with community-based indices, it is possible to discriminate and identify stressors that are affecting communities from those that are impairing key physiological functions of benthic species. Such an approach has been used in the Llobregat River to characterize the effect of habitat quality and water pollutants in river biota [19, 23, 59]. Furthermore, the use of multivariate tools, such as Principal Component (PCA) and Multivariate Partial Least Square Projections to Latent Structures regression analyses (PLS), allowed identifying specific stressors affecting the studied organisms. In the study of Barata et al. [23] latter reevaluated by Damásio et al. [19], caddisfly larvae of *Hydropsyche exocellata* were sampled from seven

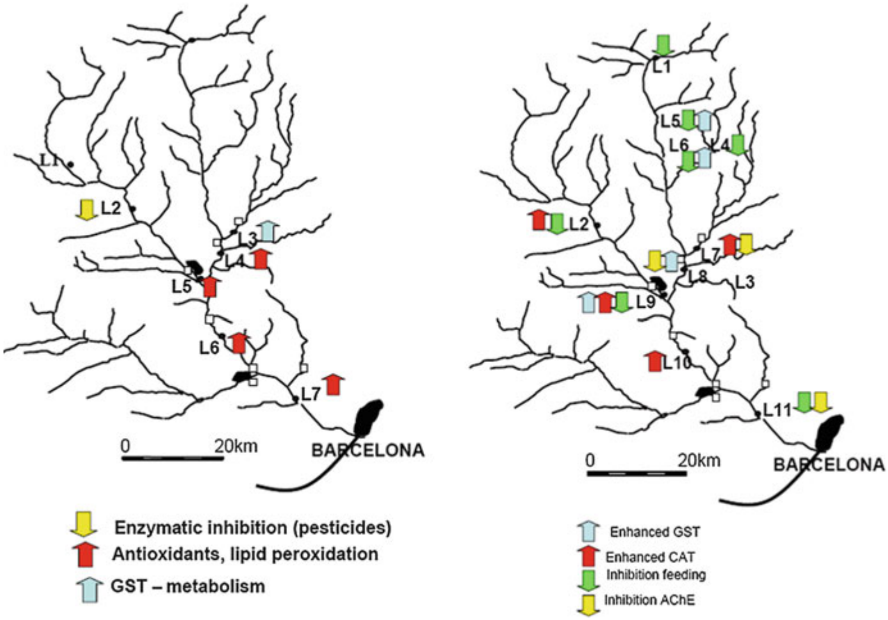

Fig. 6 Biomarker analysis in the Llobregat River. Samples were collected downstream of the main urban and industrial wastewater discharges. (**a**) Main results of *Hydropsyche exocellata* responses are superimposed to sites as *arrows* that show the direction of response compared to reference sites. Additional information can be found in [19, 23]. (**b**) Results of transplanted *Daphnia magna* individuals are superimposed to sites as *arrows* that show the direction of response compared to reference sites. Additional information can be found in [59]

locations in spring and summer receiving increasing levels of urban and industrial waste water discharges along the Llobregat River during spring and summer 2003 (Fig. 6a). Locations were selected to include aquatic communities in poor and good ecological state according to measured physicochemical water parameters and the analysis of benthic macroinvertebrate communities. Contaminant levels in water were determined in conjunction with antioxidant enzymes (superoxide dismutase, SOD; catalasa, CAT; glutathione peroxidase, GPx), a phase II enzyme (glutathione-*S*-transferase, GST) and lipid peroxide levels measured as thiobarbituric reactive species (TBARs), with the aim of investigating whether resident macroinvertebrate benthic species were responsive to changes in water quality. Metals such as Cu may also produce reactive oxygen species by redox cycling. The increment of reactive oxygen species alter the redox status of cells and antioxidant defenses: the enzyme SOD aid cells converting superoxide ions (O^{2-}) into hydrogen peroxide, catalasa, and glutathione peroxidase convert superoxides of hydrogen to water. Another set of enzymes, such as the GST, aid cells to eliminate contaminants by conjugating glutathione with contaminant metabolites generated by phase I enzymes. If production of reactive species is greater than their removal, tissue damage occurs. One marker of such tissue damage is the presence of lipid peroxidation levels.

The results of this study showed increased levels of pollutants downstream and enhanced activity levels of two (CAT and GST) out of the four tested enzymes, coupled with increased levels of lipid peroxidation measured as TBARs, indicating increasing levels of stress in the studied species toward downstream reaches or locations nearby industrial and urban areas (Fig. 6a). PCA on biomarker responses (Fig. 7) separated upstream from downstream sites, the latter having elevated levels of catalasa and lipid peroxidation. PCA on biomarkers, ecological quality indexes, and environmental factors showed that salinity and habitat quality were those

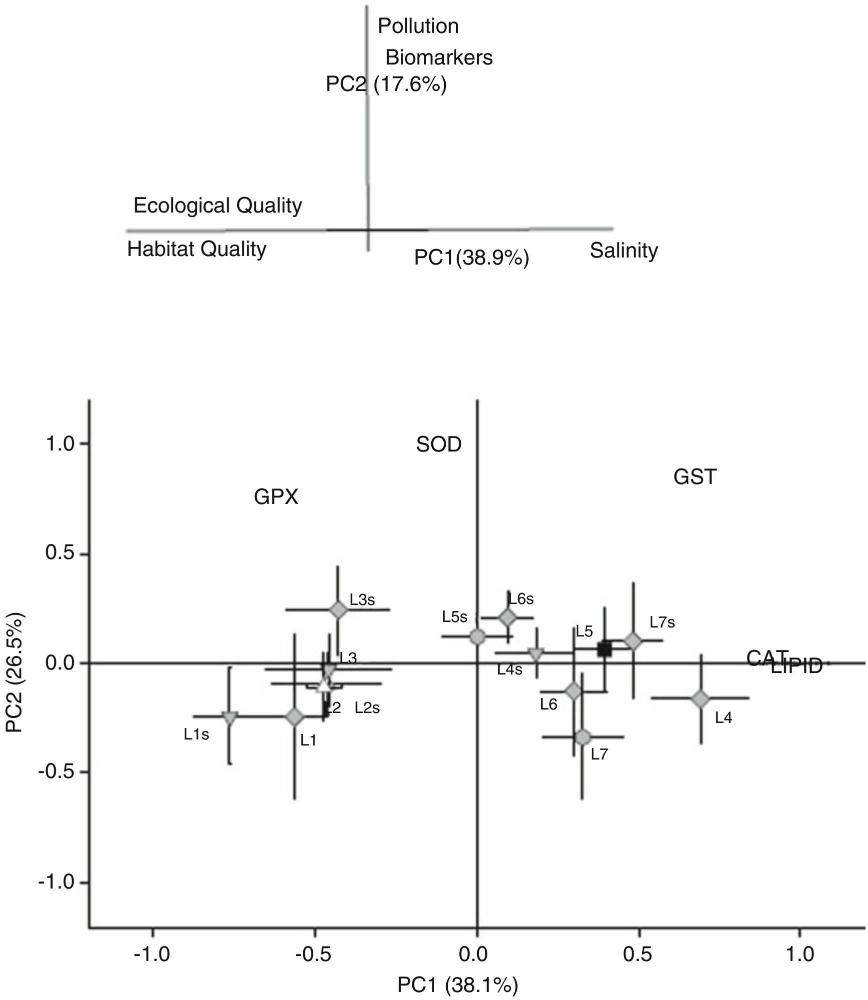

Fig. 7 PCA analysis results performed on biomarker responses. The means and 95% CI of site loadings are shown (*bottom graph*) together with the PCA results of environmental and biological variables (*top graph*). Additional information can be found in [19, 23]

factors affecting most macroinvertebrate water quality indexes, whereas pollutants did so to biomarker responses of *H. exocellata*. In resume these data indicate that the use of biomarker responses in *H. exocellata* allowed a complementary classification of water quality based on physiological stage of representative organisms rather than in whole community composition. These tools thus could be used in the future to complement currently used WFD biological indices.

In Fig. 6b we present data on a second study aimed to characterize environmental hazards of pollutants using transplanted *D. magna* individuals and determining individual (survival, feeding inhibition effects) and biomarkers [59]. This time biomarkers included the antioxidant enzyme catalasa, the metabolizing one GST and cholinesterasa, which is specifically inhibited by organophosphorous pesticides. The use of transplants using lab species allow to discard historical effects of adaptation to pollutants as well as to increase the number of sites since the presence of a particular species for sampling is not relevant. The results obtained also showed a clear deterioration of their ecological water quality parameters and benthic communities toward downstream reaches. In all but one location (L3) studied, transplanted organisms evidenced effects in at least one of the five measured responses in spring or summer. In 7 out of the 11 studied sites, significant effects were detected in at least two traits.

In two other studies, transplants and biomarkers were used to study specific effects of pollutants. *Daphnia magna* transplants and *H. exocellata* biomarkers were used to investigate potential effects of the application of the herbicide Herbolex (Aragonesas Agro, S.A., Madrid, Spain), which has glyphosate as active ingredient, to control giant reed (*Arundo donax*) [60]. Glyphosate is intended to be used in the Llobregat to eliminate foreign riparian vegetation and hence to restore autochthonous riparian vegetation. Just before and after application of glyphosate, *D. magna* individuals were deployed upstream and downstream the treated location and caddisfly larvae collected. The treated location was situated close to L10 according to Fig. 6b. Effects on benthic macroinvertebrate structure assemblages were also monitored. Measured glyphosate levels in river water following herbicide application were quite high (20–60 μg/L), with peak values of 137 μg/L after 3 days. After 12 days of its application, leaching of glyphosate from sprayed riverbanks was quite high in pore water (20–85 μg/L), but not in the river. Closely linked with the measured poor habitat and water physicochemical conditions, macroinvertebrate communities were dominated by taxa tolerant to pollution and herbicide application did not affect the abundance or number of taxa in any location. Nevertheless, only significant specific toxic effects on transplanted *D. magna* and field collected *H. exocellata* were observed. Effects included *D. magna* feeding inhibition and oxidative stress-related responses such as increased antioxidant enzyme activities related with the metabolism of glutathione and increased levels of lipid peroxidation.

In a more recent study founded by the Catalan Water Agency [61] biomarker responses of the caddisfly larvae *H. exocellata* were used to evaluate the effects of reclaimed wastewater on the ecological status of the lower part of Llobregat River, assessing if the introduction of reclaimed water during a period of low water flow (reclaimed water up to 50% of the final river flow) has any additional effect on physiological responses of the caddisfly larvae *H. exocellata*. The study was conducted close to site L11 of Fig. 6b. Again the discharge of reclaimed water did not affect the composition and abundance or the dominant taxa, consequently the ecological status measured using estructural indicators was similar before and after the introduction of treated water. Nevertheless, significant specific toxic effects on field collected *H. exocellata* larvae were observed using biomarkers probably due to the increase of chlorine levels in water together with ammonia and phosphate.

5 Conclusions

A great effort to enhance the quality status in the Llobregat basin has been carried out through sewage plant construction and habitat restoration by the Catalan Water Agency and several local institutions. That has been possible due to the high amount of information available on water quality and biological community composition, and chemical and bioassessment studies mainly provided by research centers and water authorities which have analyzed the quality status and biota in the Llobregat basin since long ago [3–5, 7, 9, 20, 23, 41]. The quality and abundance of such information have been a key element to fulfill the challenge to improve the Llobregat ecological status, and to establish a suitable monitoring program. The Llobregat River suffers a considerable ecological impact basically due to human pressures throughout the river basin [3, 6, 20]. The most important anthropogenic impacts within the Llobregat basin included salt mine activities, hydropower water diversion, and flow regime alteration by dams in headwaters and mid-Llobregat basin, together with urban and industrial sewage discharges mainly located downstream [22]. Some programs of measures have been progressively applied along time in order to mitigate such impacts. A total of 63 sewage treatment plants have been built in the last 25 years in order to reduce urban and industrial discharge impacts, and also salt runoff control has been set out around mine activities. Quality status has progressively enhanced and some chemical parameters have been reduced downstream. Ammonia concentration and, in general, nutrient loads decreased during the last decade in mid and lower Llobregat River. Such amelioration has allowed restoring biological communities, mainly diatoms and macroinvertebrates downstream, even close to the river mouth in wet periods. However, some anthropogenic pressures are still remaining and the Llobregat's biological status is not completely restored along the river. The high amount of small weirs and hydropower water diversion along Llobregat and Cardener Rivers, together with flow regime regulation by dams, riparian degradation, and eventual peak concentrations of nutrients and salts due to mining activities, result in a poor biological quality status in mid and lower Llobregat basin, where fish communities show the highest community alteration, with a high number of nonnative species appearing. Moreover, the high industrial concentration and urban discharges in mid and lower Llobregat River cause the detection of some priority substances and emergent pollutants (e.g., endocrine disruptors, heavy metals, pesticides, brominated flame retardants, drugs, and pharmaceuticals) [17–19, 23, 59], which all together increase the ecological threats.

Quality elements (macroinvertebrate, diatoms, and fish) reveal differences among human pressure impacts along the main Llobregat watercourse, from headwaters to the river mouth. Diatoms and macroinvertebrate communities show good quality in the headwaters and in the mid-Llobregat River. Downstream salt mines the macroinvertebrate community is altered, and non-tolerant taxa disappear downstream, but the biological quality status remain between moderate to good in wet periods when higher minimum flows reduce salt concentrations. The low condition of the fish fauna is related to the high habitat disturbances downstream the dams, that basically cause hydromorphological alterations, low environmental flows, habitat loss, riparian degradation, and the abundance of nonnative species. While the Llobregat headwaters and middle reaches have a good biological quality according to macroinvertebrate and diatom communities, fish quality indices indicate a poor and bad quantity status. Downstream the salt mines and in the area or large concentration of urban and industrial activities, close to the river mouth, all indices show low values. Consequently, additional analytical and biological studies have been implemented in the middle and lower Llobregat course to identify specific pressures. Chemical risk assessment studies have established the impacts of emerging contaminants, such as surfactants, pharmaceuticals, and pesticides, on local biota [17–19, 23, 59]. Biomarkers also inform us of the actual ecological status when used together with community indices, though their results are sometimes difficult to interpret [62]. It is problematic to determine whether a single biomarker response is indicator of impairment or is part of the homeostatic response, indicating that an organism is successfully dealing with the exposure [62]. However, the use of large set of biomarkers representing several metabolic paths overcome problems of interpretation and characterize the physiological effects of pollutants. The results presented herein also demonstrate the usefulness of biomarkers in detecting subtle changes of water quality in locations with deteriorated benthic communities. This is mainly due to the resilience of tolerant species assemblages to change and the great phenotypic plasticity of tolerance species such as *H. exocellata* to cope with stress. Indeed our results showed that *H. exocellata* is able to adjust quite rapidly its physiological mechanisms of defense to tolerate chemical inputs, such as glyphosate, salinity, and water flow changes. On the other hand, the use of transplants of laboratory sensitive species such as those of *D. magna* may also allow standardizing field assays. Such field assays are experimentally robust and reliable. Biomarkers should be considered in the future as tools for implementation of the WFD, in addition to community indices [63, 64]. By 2020, EU member states will have to improve the quality of their surface waters and report those changes to the WFD. In this sense, the use of markers sensitive to water pollution may provide useful information on small changes in ecological quality especially in the threshold between moderate and good.

Acknowledgments Main information on quality status, biological data, and chemical values has been obtained from the Catalan Water Agency through its monitoring program. Additional and useful historical data and biomonitoring results over time (since 1994) have been provided by the FEM research group (Department of Ecology, University of Barcelona). Historical surveillance data since 1994 were possible due to financial support provided by the Diputació de Barcelona.

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