The Handbook of Environmental Chemistry 42

Series Editors: Damià Barceló · Andrey G. Kostianoy

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Antoni Ginebreda
Narcís Prat *Editors*

Experiences from Surface Water Quality Monitoring

The EU Water Framework Directive Implementation in the Catalan River Basin District (Part I)

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Founded by Otto Hutzinger

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Aims and Scope

Since 1980, *The Handbook of Environmental Chemistry* has provided sound and solid knowledge about environmental topics from a chemical perspective. Presenting a wide spectrum of viewpoints and approaches, the series now covers topics such as local and global changes of natural environment and climate; anthropogenic impact on the environment; water, air and soil pollution; remediation and waste characterization; environmental contaminants; biogeochemistry; geo-ecology; chemical reactions and processes; chemical and biological transformations as well as physical transport of chemicals in the environment; or environmental modeling. A particular focus of the series lies on methodological advances in environmental analytical chemistry.

Series Preface

With remarkable vision, Prof. Otto Hutzinger initiated *The Handbook of Environmental Chemistry* in 1980 and became the founding Editor-in-Chief. At that time, environmental chemistry was an emerging field, aiming at a complete description of the Earth's environment, encompassing the physical, chemical, biological, and geological transformations of chemical substances occurring on a local as well as a global scale. Environmental chemistry was intended to provide an account of the impact of man's activities on the natural environment by describing observed changes.

While a considerable amount of knowledge has been accumulated over the last three decades, as reflected in the more than 70 volumes of *The Handbook of Environmental Chemistry,* there are still many scientific and policy challenges ahead due to the complexity and interdisciplinary nature of the field. The series will therefore continue to provide compilations of current knowledge. Contributions are written by leading experts with practical experience in their fields. *The Handbook of Environmental Chemistry* grows with the increases in our scientific understanding, and provides a valuable source not only for scientists but also for environmental managers and decision-makers. Today, the series covers a broad range of environmental topics from a chemical perspective, including methodological advances in environmental analytical chemistry.

In recent years, there has been a growing tendency to include subject matter of societal relevance in the broad view of environmental chemistry. Topics include life cycle analysis, environmental management, sustainable development, and socio-economic, legal and even political problems, among others. While these topics are of great importance for the development and acceptance of *The Handbook of Environmental Chemistry,* the publisher and Editors-in-Chief have decided to keep the handbook essentially a source of information on "hard sciences" with a particular emphasis on chemistry, but also covering biology, geology, hydrology and engineering as applied to environmental sciences.

The volumes of the series are written at an advanced level, addressing the needs of both researchers and graduate students, as well as of people outside the field of "pure" chemistry, including those in industry, business, government, research establishments, and public interest groups. It would be very satisfying to see these volumes used as a basis for graduate courses in environmental chemistry. With its high standards of scientific quality and clarity, *The Handbook of Environmental Chemistry* provides a solid basis from which scientists can share their knowledge on the different aspects of environmental problems, presenting a wide spectrum of viewpoints and approaches.

The Handbook of Environmental Chemistry is available both in print and online via www.springerlink.com/content/110354/. Articles are published online as soon as they have been approved for publication. Authors, Volume Editors and Editors-in-Chief are rewarded by the broad acceptance of *The Handbook of Environmental Chemistry* by the scientific community, from whom suggestions for new topics to the Editors-in-Chief are always very welcome.

Damià Barceló
Andrey G. Kostianoy
Editors-in-Chief

Foreword

Environmental quality is going to be a crucial issue for the people in charge of public affairs in the next years. To manage an environment where air, water, and soil should be in good conditions is not only an objective but a compulsory requirement in terms of well-being and of public health.

From older times water management has been a very important issue, but recently, water managers have had to cope with new challenges arising from social demands mainly focused on ecological improvement. Flowing water in rivers, lakes, estuaries, coastal waters, or reservoirs is not only regarded as resource but as a key element for sustaining aquatic ecosystems and services they provide. Good ecological status meets services and goods sustaining human well-being as well as suitable freshwater quality for safety human uses. To take into account aquatic ecosystems, preservation requires building stronger linkages between ecological, economic, and social demands with the purpose of improving water management. This framework offers the most promising way forward for the field of conservation together with a suitable human development.

Nevertheless, this challenge requires changes. Thus, in the legal side, new laws, directives, etc., are needed and institutional changes and new administrative models (development of new agencies, water authorities) are necessary. On the other side, developing new monitoring programs in order to provide suitable and enough information on water status under an ecological integrative perspective is required. Also, water management plans should be developed which encompass a comprehensive water management combining sustainable human use together with good ecological status, economic sustainability (cost recovery strategies), and social participation. Moreover, climate change should be also considered which demonstrates the scope and complexity of this challenge.

The above mentioned target makes necessary the development of new monitoring tools for water quality assessment adapted to water ecosystem types and new quality elements must be measured. Therefore, there has been a rapid increase in the development and application of ecological indicators for water quality assessment and management in developed countries. For instance, the United States, Canada, Europe, and Australia have been developing new water monitoring programs based on biological and ecological indicators for water management purposes and planning. In the European Union (EU), the Water Framework Directive (WFD) (2000/60/EC) launched in 2000 a new framework for the protection of groundwater and inland and coastal waters. The WFD represents an opportunity for a new water resource management in Europe based on ecological and economical sustainability, with the requirement of a wide social involvement. The WFD was an important conceptual change of the way that EU Member States (MS) should consider water management by putting ecosystem integrity at the base of management decisions. Since then, all MS expended considerable time and resources to collect appropriate biological, environmental, and human pressure data to develop operative tools aiming at elaborating new monitoring programs and innovative river basin management plans. As the magnitude and difficulties of this large-scale endeavor became evident, both the European community and individual MS have funded a large number of research projects, particularly in the areas of ecological assessment for water management, to develop and improve the expert knowledge. The WFD was relevant for its innovativeness and the shift towards measuring the status of all surface and coastal waters using a range of biological communities rather than the more limited aspects applied so far.

In Catalonia, the government has been deeply involved on all this process and has been implementing the WFD soon after it was adopted. Hence the administrative institution especially devoted to water management, the Catalan Water Agency (ACA), was created in 2000. ACA is in charge of planning and carrying out water management strategies in Catalonia, taking into account both water demands and environmental protection. The ACA is nowadays in charge of building and maintaining urban wastewater treatment plans, water supply management, flooding protection plans, etc. Moreover, it has been monitoring all aquatic ecosystems, including inland and coastal waters and groundwater relationship, and has been developing new tools to ensure ecological and chemical status measurements in surface waters and chemical and quantitative status in groundwater, in accordance with the WFD requirements. Additionally, some research institutes have also been promoted mainly focused on water management. An example of this is the Catalan Institute for Water Research (ICRA), that focuses its research lines in the integral water cycle, hydraulic resources, water quality (in the broadest sense of the term: chemical, microbiological, ecological, etc.), and treatment and evaluation technologies. The research carried out at the ICRA has to do with all the aspects related with water, particularly those associated with its rational use and the effects of human activity on hydraulic resources.

Over the last decades, it has been necessary to monitor and to assess the ecological status of water bodies following the WFD guidelines. Accordingly, the ACA started a close science to policy relationship with research institutions, which have been closely involved in such development. From this collaboration novel methodologies have been proposed, and a huge amount of data has been gathered over more than a decade. Overall, this cooperation has proved to be a stimulating and fertile ground for research of the interface between science and management. Accordingly, the Catalan Water Agency (ACA) established a new monitoring program in order to provide a proper water status diagnosis just before the water management plan's updating in the Catalan River Basin District. The ACA has now a global picture of the ecological and chemical status of all water bodies in Catalonia. The experience gained by the Agency over the last 15 years has been incorporated in these two different book volumes that I have the privilege to introduce in this preface: *Experiences from Surface Water Quality Monitoring: The EU Water Framework Directive Implementation in the Catalan River Basin District (Part I)* and *Experiences from Ground, Coastal and Transitional Water Quality Monitoring: The EU Water Framework Directive Implementation in the Catalan River Basin District (Part II)*. Both books summarize all the findings on water monitoring for WFD purposes, and they discuss further perspectives according to the new knowledge obtained. They are devoted to such effort which has resulted in a series of protocols adapted to the aquatic ecosystem monitoring in Catalonia. Both books encompass several specific chapters focused on different aquatic systems (rivers, lakes, wetlands, reservoirs, estuaries, bays, coastal waters, and groundwater) and are written by several researchers in close collaboration with ACA's technicians. They provide good examples and suitable monitoring tools for aquatic ecosystem monitoring in Catalonia that can also be easily extrapolated to other Mediterranean river basin districts. Data analyzed and information obtained are not only useful in understanding the current quality status but also gathering the necessary knowledge to design the best tools for aquatic ecosystem management and restoration and/or conservation measures adapted to each aquatic ecosystem type, paying special attention to Mediterranean conditions which deeply affect water management in southern Europe. At that time, just to end I can say that we are proud of the work done by our community of experts in water management working in public administrations, in research centers, and in private companies. I hope that the materials and experiences enclosed in the two volumes reflect a step forward of a better management of water and stimulate new developments for the future.

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Volume Preface

Freshwater systems in Europe are threatened by a variety of stressors (chemical pollution, geomorphological alterations, changes in land uses, climate variability and change, water abstraction, invasive species, and pathogens). Chemical aquatic pollution today comprises a wide range of emerging chemical substances, such as pharmaceuticals, personal care products, or pesticides, among others. Stressors are of diverse nature but cause adverse effects on biological communities and ecosystems. It is well known that the relationship between multiple stressors might determine changes in the chemical and ecological status, which are the key objectives of the European Union Water Framework Directive (WFD). This important piece of legislation has pushed the EU River Basin Authorities to carry out advanced monitoring programs in collaboration with universities and research centers.

These two volumes of *The Handbook of Environmental Chemistry* we introduce here (Volume I: *Experiences from Surface Water Quality Monitoring: The EU Water Framework Directive Implementation in the Catalan River Basin District (Part I)* and Volume II: *Experiences from Ground, Coastal and Transitional Water Quality Monitoring: The EU Water Framework Directive Implementation in the Catalan River Basin District (Part II)*) correspond to an excellent collaborative example between the River Basin Authority from the Catalan River Basin District (NE Spain), the so-called Catalan Water Agency (ACA), with the Catalan Universities and Research Centers. These books cover the main research outcomes achieved during the last 10 years following WFD implementation. It contains a total of 26 chapters and over 75 authors who explain how, from the interaction between the ACA and several academic centers, the different quality elements included in the WFD have been adapted to Mediterranean aquatic ecosystems. We want to remark the importance of this interaction between the members of the ACA and the members of academia or experts in a collaborative effort that probably is unique in the WFD implementation in Europe.

Why ACA has developed such collaborative effort? First of all because for most of the biological elements, no or few experience in how to use such elements existed in Spain Water authorities. ACA had more experience in the analysis of chemical parameters, i.e., priority substances. Second, the methods to be used by WFD guidelines should be inter-calibrated; therefore ACA was aware that a set of methodologies with a robust scientific background was needed, so their results could be compared to other European countries. Third, most of the streams in Catalonia are in a Mediterranean climate area, and for this reason, taxa present in aquatic ecosystems and their environmental constraints are different from those of more temperate ecosystems from Europe. Scientifically robust methodologies should be adopted by ACA to explain why our aquatic ecosystems are different and how these differences affect the way in which the water quality is measured.

The ACA has easily found the way to build up from the scientific knowledge the tools needed by the administration to measure the status of the water. Catalonia has a long tradition on water quality studies which is grounded in the shoulders of several Masters and Commanders of Science. We think that at least two of them should be quoted: the former professors of the University of Barcelona Ramón Margalef and Enric Casassas. Margalef was a well-known ecologist and the first professor of Ecology in Spain, and Cassassas was the introductor of modern analytical techniques in Spain. In a postwar situation, after Spanish civil war (1936–1939) and the second world war (1939–1945), scientific research in Spain was very poor and many times under scientifically unreliable people. The late professors Ramon and Enric were extremely clever and open-minded people, and despite many obstacles, they found a way to put the roots of what now is one of the best schools of aquatic studies in Europe. Both were excellent professors and researchers and generous people with new ideas and solutions. Certainly they were an example of scientists with a global vision but with a local action, with a real compromise with their homeland, Catalonia. This school has produced an array of young scientists (not so young anymore) that have studied in-depth many aspects of ecology or chemistry in freshwater systems with a deep vision on the Mediterranean water bodies. At the same time, most of these students formed many other students and these to other, so the first grand-grand-children are at this moment at the front line of water quality research studies. Other masters exist also in Catalonia in hydrogeology, microbiology, or fish ecology, that several of the authors of this book have taken advantage.

Thanks to the effort of Margalef, Cassasas, and others and his students; when ACA started to think what to do for the implementation of the WFD, most of the fundamentals for such work were there. But in many cases the scientific research is not applied for the administration because the two worlds are hardly in contact. The merit to understand that such relationship is necessary should be given to some of the directors of the ACA and some of the ministers of the environment of the regional government of Catalonia who recognized the importance of such collaboration. It was of help too that some of the disciples who did their Ph.D. with students of the two masters already mentioned took a position in ACA. These people are now coeditors, with Prof. Prat, of these two books: Antoni Munné and Antoni Ginebreda. Both are Ph.D. from Catalan universities and understand that without the collaboration of scientist and managers, it is almost impossible to produce enough robust tools to be compared with other well-known tools developed elsewhere. We, the scientists, should be very aware of the role of these two people because without their effort these two books could never be produced.

We hope that this book will be of much interest for many international readers too. We think that it will be a useful guide for other European river basins, as well as in other parts of the world, as a good example of the added value of collaborative research on aquatic sciences. Indeed the books contain a comprehensive list of monitoring programs of importance for WFD implementation to the Mediterranean climate aquatic ecosystems. The literature references of the different chapters contain great amount of work produced by these numerous groups of academics and managers working and publishing together in the most relevant journals of ecology, fishes, microbiology, analytical chemistry, etc. We thank all of them for their time spent writing all the different chapters and making these books unique in this series.

We, as the most senior authors and former students of Margalef and Cassasas, are very proud of this work. We thank very much the ACA and the government of Catalonia for continuously supporting such work. We encourage as well, even under the present economic difficulties, to maintain such effort. It is obvious that new methodologies and tools will need to be incorporated to monitor programs in the future. We believe that the best way to do it is by establishing bridges of collaboration between scientist and managers.

Barcelona, Spain Narcís Prat and Damià Barceló

Contents

Water Status Assessment in the Catalan River Basin District: Experience Gathered After 15 Years with the Water Framework Directive (WFD)

Antoni Munné, Antoni Ginebreda, and Narcís Prat

Abstract The Catalan Water Agency (ACA) established a WFD Monitoring Programme for a period of 6 years (from 2007 to 2012) in order to provide a proper water status diagnosis just before the water management plan's updating in the Catalan River Basin District (NE Spain). Most of applied monitoring tools were developed over the last decade in close cooperation with research centres and universities in order to assure they are WFD compliant. Thus, novel methods arose and have been published in research papers over the last decade providing new tools for water quality monitoring and bioassessment in Mediterranean WB.

Using all this experience, a comprehensive monitoring network has been established by the ACA to provide a coherent overview of both ecological and chemical status for surface waters and chemical and quantitative status for groundwater. Therefore, chemical and ecological status were assessed in rivers (248 WB), lakes and wetlands (27 WB), reservoirs (13 WB), transitional waters (26 WB), coastal waters (33 WB), and groundwater (37 WB). A total of 137 out of 384 WB were classified as good status (36%), whereas 221 (58%) were classified as bad. However, some uncertainties were found when applying the monitoring program which raised some concerns in the way of quality assessment and ecological status classification. A total of 59 (15%) WB classified as good and 107 (28%) WB

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classified as bad showed uncertainties when classifying between good and bad quality status, and quality status could not be finally assessed in 25 (6%) WB (temporary WB) due to difficulties found applying current protocols. Uncertainties have been highlighted in order to be solved near future.

Keywords Catalan River Basin District, Chemical status, Ecological status, Mediterranean basins, Monitoring program, Water Framework Directive

Contents

Acronyms

ACA	Catalan Water Agency (in Catalan)
BQEs	Biological quality elements
CIS	Common Implementation Strategy
CRBD	Catalan River Basin District
EQR	Ecological quality ratio
EU	European Union
GWD	Groundwater Directive (2006/118/EC)
NBLs	Natural background levels
QS	Quality standard
TVs	Threshold values (to set water quality classes)
WB	Water bodies
WFD	Water Framework Directive (2000/60/EC)

1 Introduction

The publication of the Water Framework Directive (2000/60/EC) (WFD) by the European Parliament and the Commission at the end of 2000 [1] and the subsequent adopted daughter Directives (Groundwater Directive 2006/118/EC and Directives 2008/105/EC and 2013/39/EU on priority substances) published between 2006 and 2013 [2–4] have promoted the integrated water quality assessment of aquatic ecosystems across Europe [5–7]. Water managers have had to cope with the new challenges arising from the Water Framework Directive requirements [8]. Flowing water in rivers or water from lakes, estuaries, coastal waters or reservoirs should not only be regarded as resource but as a key element for sustaining the aquatic ecosystem life and services they provide. Good ecological status is required to meet services and goods for human well-being, as well as suitable freshwater quality for human uses. Therefore, biological communities which should naturally inhabit those ecosystems emerged as key elements (biological quality elements – BQEs) together with chemical and habitat status in order to enhance ecosystem's health diagnosis and the accomplishment of the WFD objectives. Therefore, new approaches are required to meet this target, which implies the development of suitable biomonitoring protocols and specific monitoring networks for each European Basin District. EU member states were bound to apply such monitoring since 2007 according to the WFD. Accordingly, water quality measurements using biological quality elements (fish, macrophytes, fitobenthos, macroinvertebrates, etc.) and hydromorphological conditions must be incorporated to monitoring programmes together with physicochemical parameters already used in most European countries so far. Thus, we can finally establish the status of all WB (rivers, lakes, reservoirs, wetlands, estuaries, coastal lagoons, coastal waters, etc.) by combining all quality elements. Additionally, groundwater must also be considered as a part of water cycle and thus monitored [9, 10]. Many surface ecosystems are closely related to groundwater, and water quality and quantity of subterranean waters must be taken into account when ecological status is measured.

These new challenges made necessary the development of new monitoring tools adapted to each river basin district and ecosystem types [8, 11, 12]. Additionally, climate constraints must also be taken into account, especially in Mediterranean areas where water flow is often scarce and irregular along time. Several studies have focused on the biological communities that live in this kind of aquatic systems (reviewed in Lake [13]). However, often those singularities have not been fully integrated into water policy because most water managers tend to apply perennial river management principles to temporary ones, when new tools are required to be adopted in this kind of aquatic ecosystems [14, 15]. This is the case of the Catalan basins (NE Spain), in which the implementation of the WFD has required a collaborative work between research centres, water authorities, and many stakeholders in order to achieve such targets. This manuscript is devoted to such effort, which results in a series of protocols adapted to a Mediterranean River Basin District (the Catalan basins, NE Spain).

The WFD implementation in Spain has been led through the Spanish Government, but the necessary works for this implementation have been carried out by the water authorities in charge of water management at basin scale (River Basin Authorities). In Spain, the territory managed by the River Basin Authorities is not often coincident with political divisions (autonomous regions), which has led to a relative complex system of water management. Most of the largest basins in Spain have to be governed by a Committee of Water Authorities with representatives of the autonomous regions with all or part of its territory in the basin (intercommunity basins). However, when the River Basin District is totally located within a single political territory unit or region (intracommunity basins) water management is undertaken by the autonomous government of that region. This is the case of the Catalan River Basin District (CRBD), an intracommunity basin with a total area of 16,438 km^2 made up by several small- to medium-sized basins totally located within the Catalan region (Fig. 1), which is managed by the Catalan Water Agency

Fig. 1 The Catalan River Basin District (CRBD) (NE Spain), which occupies half part of the whole Catalan territory, is shown in grey. The other half part of Catalonia is part of the Ebro river basin district

(ACA). The Catalan River Basin District (CRBD) occupies half part of the whole political limits of Catalonia (NE Spain). Catalonia has a total area of 32,114 km^2. The other half part of Catalonia is occupied by a small part of a much larger watershed, the Ebro basin (15,676 km^2 out of 85,660 km^2 of total Ebro basin area), an intercommunity basin. However environment policy, fishing management, and urban and agricultural planning are in hands of Catalan autonomous government in all Catalonia, even in the Catalan part of the Ebro basin. Additionally, the Catalan Water Agency also manages water supply and urban waste water treatment plants in the whole Catalan region. Applying WFD to Catalan territories became, due to this dichotomy, more complicated. Therefore, while the Catalan Water Agency (ACA) applies monitoring programs and planning decisions in the Catalan River Basin District, in the Catalan part of the Ebro watershed, ACA offers support and monitoring data, but water planning decisions are in the hands of the Ebro River Basin Authority (Confederación Hidrográfica del Ebro).

Under this framework, and regardless of those two different water policy situations in Catalonia, the Autonomous Government of Catalonia has been implementing the WFD in the whole Catalan region. Hence, the Catalan Government created the ACA in 2000 which is charge of the planning and maintenance of urban wastewater treatment plans, drinking water supply management, monitoring and water status surveillance, flood protection, etc. The ACA has been monitoring all aquatic ecosystems around Catalonia (including coastal waters) since the year 2000 and has been developing a system to ensure that ecological and chemical status measurements in the whole Catalan WB are in accordance with the WFD requirements. All quality elements required by the WFD have been studied and detailed sampling protocols developed. Monitoring and data acquisition and treatment have been implemented over the last decades following the WFD requirements. Several research institutions have been closely involved in such development and, in many cases, novel methods have been raised and published. A huge amount of data has been gathered over more than a decade through the development of several research projects specially supported by ACA. Most relevant and recent information gathered from hundreds of research papers and reports produced by many institutions may be found in Table 1. Therefore, the ACA has now a global picture of the ecological and chemical status of Catalan WB. In this chapter we synthesized such knowledge and analysed the data not only to understand the current situation or quality status of Catalan aquatic ecosystems but also gather the necessary knowledge to design the best restoration and/or conservation measures adapted to each river type present in its territory. The experience gained over the last 15 years has been incorporated in two different books: "Experiences from Surface Water Quality Monitoring. The EU Water Framework Directive Implementation in the Catalan River Basin District (Part I)" and "Experiences from Ground, Coastal and Transitional Water Quality Monitoring. The EU Water Framework Directive Implementation in the Catalan River Basin District (Part II)". In this chapter we summarize all the findings and we discuss further perspectives according to the new knowledge obtained.

Table 1 Most relevant papers published in the last decade, with the support of the Catalan Water Agency, developing or testing new tools for water quality monitoring according to the WFD

Water category	Research topic relevant to WFD implementation	Related references
Rivers	Diatoms	[16–18]
	Macroinvertebrates	[19–21]
	Macrophytes	[22, 23]
	Fish and river connectivity	[24–27]
	Reference conditions	[28]
Reservoirs	Typology	[29]
	Fish	[30]
Lakes and wetland	Invertebrates	[31]
	Ecohydrology	[32]
Transitional waters and coastal lagoons	Invertebrates	[33–35]
	Diatoms	[36, 37]
Transitional waters – Ebro Delta Bays	*Cymodocea nodosa*	[38]
Coastal waters	Phytoplankton	[39, 40]
	Macroalgae	[41–44]
	Posidonia oceanica	[45–49]
	Macroinvertebrates	[50–52]
Groundwater	Polar pesticide analysis	[53, 54]
	Pharmaceuticals and nitrate source tracking	[55, 56]
	Hydrogeochemical tools	[57]

2 The Water Quality Assessment in the Catalan WB

2.1 General Remarks

Although there was a long tradition of chemical and biological monitoring in Catalan rivers, lakes, reservoirs, coastal lagoons, coastal waters, and groundwater (see some examples in [58–63]), protocols and biological quality indices WFD compliant were not completely available when the WFD was adopted. The ACA launched a programme of science to policy relationship with several research centres and universities in order to provide suitable knowledge on all the biological, hydromorphological, and chemical elements necessary to meet the WFD targets for ecological status assessment. Most of such research works (i.e. [64–66]) were used to enhance monitoring protocols and the ecological status assessment methods. Indices need to be properly tested and correlated with a stressor gradient for major human pressures in order to provide a useful and coherent tool for water quality diagnosis comparable among watersheds and regions [8]. For this reason ACA has taken part in the Mediterranean Geographical Intercalibration Group (Med-GIG) since 2006 in order to harmonize and intercalibrate the information obtained [8, 67–70]. The intercalibration exercise ended in formal decisions published by the

European Commission in 2008 and 2013 [69, 71]. Groundwater quality elements were also analysed according to the guidelines provided by the CIS working group [9, 10]. Regarding chemical status, it has been also established by the compliance with the environmental quality standards (EQS). A total of 97 priority substances and group of substances (isomers, metabolites, etc.) are currently analysed by the Catalan Water Agency using standard procedures [72].

Therefore, ACA has developed a monitoring program to assess quality status for all Catalan WB according to the EU-WFD requirements and following the intercalibration process in close cooperation with research centres. Such monitoring program and its main features are available in the Catalan Water Agency WEB page (www.gencat.cat/aca).

2.2 Monitoring Networks

The ACA established the first WFD Monitoring Programme for a period of 6 years (from 2007 to 2012). The entire monitoring program is completed after 6 years according to the WFD. Several reports on water quality status were produced over this period (www.gencat.cat/aca). A reviewed second Monitoring Programme was launched in 2013, for an additional 6 years (from 2013 to 2018) which is currently in progress. Details of both monitoring programmes are available in the Catalan Water Agency web page (www.gencat.cat/aca). Both monitoring programmes have been providing enough data to establish the ecological status diagnosis according to the WFD requirements.

Monitoring networks were designed to provide a comprehensive spatial overview of both ecological and chemical status for surface waters and chemical and quantitative status for groundwater. Monitoring networks were established for each quality element taken into account intensity of human pressures in the territory (Tables 2, 3, and 4). Different sampling frequencies were applied for each sampling site considering each quality elements and the intensity of human pressures (Table 5). For example, fish are scheduled to be sampled once over a period of 6 years in all river water bodies, whereas macroinvertebrates have been sampled every year in WB affected by intense human pressures but twice for a 6 years period in pristine or slightly altered WB. General chemical parameters (e.g. nutrients, ions, salinity, pH, etc.) have been sampled monthly in river WB at risk but every 3 months when the human pressures are slight or null, etc. (Table 5).

Regarding Groundwater Monitoring Program, it comprises 1,035 sampling sites in 39 ground WB. The density fluctuates from 1.14 sampling sites per 10 km^2 to 0.25 sampling sites per 10 km^2 depending on the high or low intensity of human pressures, respectively, and the well availability. A total of 577 sites are used for chemical surveillance monitoring, from which 279 are also used for nitrate operational monitoring, 84 for pesticide operational monitoring, 183 for salinity in coastal areas, 239 for industrial risk operational monitoring, 476 for nitrate vulnerable protected areas, and 138 sampling sites to follow the quality of water

Table 2 Number of sampling sites for each water body category and monitoring networks in the Catalan River Basin District (16,438 km^2)

	Surveillance monitoring		Operational monitoring					Protected areas monitoring	
Water body category	Chemical status	Quantitative status	Areas with high risk to be affected by nitrate	Areas with high risk to be affected by pesticides	Shore areas with high risk to be affected by salinity (marine intrusion)	Areas affected by industrial activities	Areas affected by mine activities	Vulnerable zones by nitrate 91/676/EC Directive	Areas for drinking water supply
Groundwater	577	207	279	84	183	239	11	476	138

Monitoring networks are grouped by surveillance or operational or for protected areas.

Table 3 Number of sampling sites for each water body category and monitoring networks in the Catalan River Basin District (16,438 km^2)

Water body category	Surveillance monitoring: Chemical status	Surveillance monitoring: Ecological status	Operational monitoring: WB highly affected by human pressures	Operational monitoring: WB affected by priority substances (water + sediment + biota)	Operational monitoring: Areas affected by mine activity	Protected areas monitoring: Vulnerable zones by nitrate 91/676/EC Directive	Areas for drinking water supply	Sensitive areas 91/271/EC Directive	Bathing water areas 2006/7/EC Directive	Fish life 2006/44/EC Directive	92/43/EEC and 2009/147/EC Directives
Rivers	211	248	111	39 + 24 + 26	15	187	37	83	4	19	136
Reservoirs	13	13	5	5 + 2 + 2	–	13	5	6	5	–	8
Lakes	27	29	22	15 + 0 + 0	–	15	1	2	2	–	18
Transitional waters (coastal lagoons)	25	28	22	22 + 0 + 0	–	12	–	7	–	–	23

Monitoring networks are grouped by surveillance or operational or for protected areas.

Table 4 Number of sampling sites for each water body category and monitoring networks in the Catalan River Basin District (16,438 km^2)

Water body category	Surveillance monitoring			Operational monitoring			Protected areas monitoring				
	Chemical status (organic compounds + heavy metals)		Ecological status (phytoplankton + macroalgae + phanerogams + macroinvertebrates)	WB highly affected by human pressures	WB affected by priority substances (organic compounds + heavy metals)		Vulnerable zones by nitrate 91/676/EC Directive	Areas for drinking water supply	Sensitive areas 91/271/EC Directive	Bathing water areas 2006/7/EC Directive	92/43/EEC and 2009/147/EC Directives
	In water	In sediments			In water	In sediments					
Coastal waters	32 + 29	94 + 47	87 + 36 + 29 + 37	23	16 + 16	52 + 30	51	2	1	231	29

Monitoring networks are grouped by surveillance or operational or for protected areas.

Table 5 Number of samples per a 6 year period in the ACA Monitoring Programme (2013–2018) carried out in the Catalan River Basin District

	Biological elements					Chemical and physicochemical elements				Hydromorphology conditions
Water body category	Macroinvertebrate	Fitobenthos (diatoms or macroalgae)	Macrophytes or phanerogams	Fish	Phytoplankton	General pollutants	Priority substances on water	Priority substances on sediment	Priority substances on biota	Hydrology – connectivity – morphology
Rivers	HPWB: 6	HPWB: 6	HPWB: 1	HPWB: 1	–	HPWB: 72	HPWB: 24	HPWB: 6	HPWB: 6	HPWB: 1
	WPWB: 2	WPWB: 2	WPWB: 1	WPWB: 1		WPWB: 24	WPWB: 2	WPWB: 0	WPWB: 0	WPWB: 1
	RWB: 2	RWB: 2	RWB: 1	RWB: 1		RWB: 24	RWB: 22	RWB: 0	RWB: 0	RWB: 1
Reservoirs	–	–	–	HPWB: 1	HPWB: 6	HPWB: 6	HPWB: 3	HPWB: 1	HPWB: 1	–
				WPWB: 1	WPWB: 3	WPWB: 3	WPWB: 2	WPWB: 1	WPWB: 1	
				RWB: 1	RWB: 3	RWB: 3	RWB: 1	RWB: 0	RWB: 0	
Lakes and wetlands	HPWB: 2	–	HPWB: 2	–	NAY	HPWB: 3	HPWB: 3	HPWB: 1	–	HPWB: 2
	WPWB: 2		WPWB: 2			WPWB: 2	WPWB: 2	WPWB: 1		WPWB: 2
	RWB: 2		RWB: 2			RWB: 2	RWB: 2	RWB: 0		RWB: 2
Transitional waters	HPWB: 2	–	HPWB: 2	–	NAY	HPWB: 3	HPWB: 3	HPWB: 1	–	HPWB: 2
	WPWB: 2		WPWB: 2			WPWB: 2	WPWB: 2	WPWB: 1		WPWB: 2
	RWB: 2		RWB: 2			RWB: 2	RWB: 2	RWB: 0		RWB: 2
Coastal waters	HPWB: 2	HPWB: 3	HPWB: 3	–	HPWB: 48	HPWB: 48	HPWB: 12	HPWB: 6	–	NAY
	WPWB: 2	WPWB: 3	WPWB: 3		WPWB: 24	WPWB: 24	WPWB: 6	WPWB: 1		
	Biological elements					**Chemical elements**				**Water quantity**
Water body category	**Macroinvertebrate**	**Fitobenthos (diatoms or macroalgae)**	**Macrophytes or phanerogams**	**Fish**	**Phytoplankton**	**General pollutants**	**Salinity**	**Nitrate**	**Pesticides**	**Piezometric level**
Groundwater	–	–	–	–	–	HPWB: 6	HPWB: 6	HPWB: 6	HPWB: 6	HPWB: 72
						WPWB: 3	WPWB: 0	WPWB: 0	WPWB: 0	WPWB: 72

The number of scheduled samples is shown for several groups or types of WB: *HPWB* highly pressured WB, WPWB weakly pressured WB, *RWB* reference WB, *NAY* not applied yet withdrawals for drinking human supply (Tables 2, 3, and 4). Additionally, 11 sampling sites are solely monitored for salt mine activity surveillance), and 207 sites are exclusively used for water-level surveillance.

A total of 248 sampling sites are set for river surveillance purposes (one per each water body), using different sampling frequencies depending of the element to be measured (see Tables 2, 3, 4, and 5). Additional sampling sites have been established from protected areas (i.e. quality control for drinking protected areas or bathing zones) or for particular purposes. For example, 26 sampling sites were included to measure reference conditions of different river types, or 4 sampling sites are exclusively used for bathing monitoring. Regarding reservoirs, a total of 13 sites have been established in 13 reservoirs. Moreover, five additional sites are set to exclusively provide water supply quality data for urban uses, and an additional five sampling sites were set to monitor bathing areas (protected areas) (see Tables 2, 3, and 4). Wetlands and karstic lakes (lakes) and coastal lagoons (transitional waters) have also been analysed with a total of 57 sites, one sampling site per each water body. The same sites are used to obtain different information (Table 5). Finally, coastal waters, like groundwater, are monitored using several sampling sites per each water body with a total of 87 sampling sites for physicochemical parameters and phytoplankton (chlorophyll-a) in 33 coastal WB, 54 near coastline, and 33 offshore. Moreover, additional 36 sampling sites are used to evaluate macroalgae, 29 for *Posidonia oceanica*, and 37 sites to assess benthic macroinvertebrates quality indices (Tables 2, 3, and 4). So a total of 574 sites are set in 33 WB, but unlike inland waters, not all parameters are collected from the same sampling site.

2.3 Sampling Protocols and Selected Metrics

Detailed protocols for sampling and metrics used for biological and hydromorphological quality assessment can be found in the Catalan Water Agency web page (https://aca-web.gencat.cat). A summary is provided in Table 6. As previously said, most of biological quality elements applied in rivers and coastal waters of the Catalan Water District were intercalibrated through the European Commission process carried out from 2006 to 2013 [71].

Chemical status in surface waters is assessed through a total of 97 priority and hazardous chemicals analysed using standard procedures [72], like atomic fluorescence spectroscopy for mercury, inductively coupled plasma mass spectrometry for metals, headspace extraction procedure for solvent substances (UNE-EN ISO 1030 1997), solvent extraction with simultaneous derivatization for pentachlorophenol [74], and solid-phase stirred bar extraction [75] for the rest of organic compounds. All chemicals were also analysed or confirmed using GC–MS according to the 2009/90/EC Directive. From these 97 substances and group of substances, 42 are included in the Annex I of the 105/2008/EC Directive [72]. Only substances and thresholds provided by the 105/2008 and 2013/39/UE Directives were applied for

Table 6 Biological quality indices currently used by the Catalan Water Agency in order to establish the ecological status in the Catalan River Basin District (rivers, reservoirs, lakes, transitional waters, and coastal waters)

Water category	Biological quality element	Biological index	Organization level based[a]	Type of index[b]	Index sensitivity[c]	Intercalibrated[d]	Information source[e]
Rivers	Diatoms	IPS	Community	Unimetric	Nutrients – general degradation	Yes. DOCE 2008	Chap. 3 (Part I)
	Macroinvertebrates	IBMWP	Community	Unimetric	General degradation	Yes. DOCE 2008	[73]
		IMMi-T	Community	Multi-metric	Nutrients – general degradation	Yes. DOCE 2013	[19]
	Macrophytes	IBMR	Community	Unimetric	Nutrients – general degradation	Yes. DOCE 2013	Chap. 4 (Part I)
		IMF	Community	Unimetric	Nutrients – general degradation	No	[23]
	Fish	IBIMED/ IBICAT	Community	Multi-metric	Nutrients – general degradation	Yes. DOCE 2013	Chap. 5 (Part I)
		IBICAT2b	Community	Multi-metric	Nutrients – general degradation	No	Chap. 6 (Part I)
Reservoirs	Phytoplankton	IGA	Community	Unimetric	Nutrients – general degradation	Yes. DOCE 2008.	Chap. 9 (Part I)
		Chl-a	Population biomass	Unimetric	Nutrients – general degradation	Yes. DOCE 2008	Chap. 9 (Part I)
		%CIANO	Popul. biovolume	Unimetric	Nutrients – anoxia	Yes. DOCE 2008	Chap. 9 (Part I)
Lakes – inland wetlands	Phytoplankton	InGA	Community	Unimetric	Nutrients	No	
	Macrophytes	InMac	Community	Unimetric	Nutrients – general degradation	No	
	Invertebrates	InMacro	Community	Unimetric	Nutrients – general degradation	No	
		QAELS	Community	Multi-metric	Nutrients – general degradation	No	Chap. 8 (Part I)
Transitional waters – coastal lagoons	Invertebrates	QAELS	Community	Multi-metric	Nutrients – general degradation	No	Chap. 8 (Part I)
		EQAT	Community	Unimetric	Nutrients – general degradation	No	Chap. 8 (Part I)

(continued)

Table 6 (continued)

Water category	Biological quality element	Biological index	Organization level based[a]	Type of index[b]	Index sensitivity[c]	Intercalibrated[d]	Information source[e]
Transitional waters – Ebro Delta Bays	Phytoplankton	Chl-a	Population biomass	Unimetric	Nutrients – general degradation	No	Chap. 6 (Part II)
	Marine phanerogams:						
	Cymodocea nodosa	CYMOX	Population and biomarkers	Multi-metric	General degradation	No	Chap. 10 (Part II)
	Macroinvertebrates	MEDOCC	Community	Multi-metric	Organic matter – general degradation	No	Chap. 9 (Part II)
Coastal waters	Phytoplankton	Chl-a	Population biomass	Unimetric	Nutrients – general degradation	Yes. DOCE 2008	Chap. 6 (Part II)
	Macroalgae	CARLIT	Community	Multi-metric	Nutrients – general degradation	Yes. DOCE 2008	Chap. 8 (Part II)
	Marine phanerogams:						
	Posidonia oceanica	POMI	Population and biomarkers	Multi-metric	Nutrients – general degradation	YES. DOCE 2013	[48, 49]
	Macroinvertebrates	MEDOCC	Community	Multi-metric	Organic matter – general degradation	Yes. DOCE 2008	Chap. 10 (Part II)

[a]Level of biological organization upon which bioindicator is based. The effects of human pressures involve a series of biological responses measured through several organization levels in aquatic ecosystems: ranking from population and community (e.g. biological indices) up to lower levels as biomolecular/biochemical levels (e.g. biomarkers)
[b]Index calculation structure and composition: unimetric/multi-metric indices or multivariant index, predictable model, etc.
[c]Main detected pressure(s). Specification of pressure-impact relationship upon which quality index was developed
[d]Was the biological index intercalibrated by the EU exercise as a Spanish national index through the Mediterranean GIG?
[e]Sources where additional information can be found for each quality element or index applied in the CRBD. Both references or chapters of the the present book titled: "*Experiences from Surface Water Quality Monitoring. The EU Water Framework Directive Implementation in the Catalan River Basin District (Part I or Part II)*" are provided to find additional information

chemical status assessment. Values of heavy metals (lead, cadmium, mercury, and nickel), chlorinated solvents, pesticides (chlorine, phosphorus, triazine), polycyclic aromatic hydrocarbons, and endocrine disruptors (nonylphenols, octylphenols, and brominated diphenyl ether compounds) were analysed and compared with the EQS provided by the directives. All other chemical elements are used to analyse their evolution along time and detect possible hot spots [76].

Regarding groundwater, chemical quality standards and threshold values (TVs) were established in accordance with the procedure set out in Annex I and II of the Directive 2006/118/EC for groundwater [9, 10]. Natural background levels (NBLs) were set by calculating the 90 percentile of the historical dataset of major ions and natural chemical compounds in order to establish TVs for quality classes (between good and bad). TVs and quality classes used for ground WB can be found in the Catalan River Basin Management Plan (https://aca-web.gencat.cat). The groundwater quantitative status is measured by protocols produced by ACA and available at the same web page.

3 Quality Status in Catalan WB

Applying sampling protocols and metrics previously mentioned (Table 6), the ecological and chemical status have been assessed in all surface Catalan water body categories (rivers, reservoirs, lakes, wetlands, and transitional and coastal waters) (Fig. 2). Moreover, chemical and water level have been analysed for groundwater (Fig. 3). Water body diagnostics have been carried out together with scientific support to ensure the ecological interpretation for management purposes.

3.1 Rivers

Rivers have been analysed according to both ecological and chemical status. Biological quality has been assessed using fish, macroinvertebrate, and diatom quality elements (see Table 5). Macrophytes are not used yet as biological quality metric in rivers due to scarce data available so far. A total of 39% of river WB (97 out of 248) show high or good biological quality in the Catalan River Basin District. On the other hand, 38% of river WB show values below good quality (94 out of 248) mainly located close to urban and industrial areas. For a total of 17 WB (7%), it was not possible to assess the biological quality because of lack of data mainly due to scarce or null flow (temporary rivers) (Fig. 2). Results reveal that fish index is the most stringent biological quality element in rivers. Whereas macroinvertebrate and diatom indices (IBMWP and IPS, respectively) show quite similar percentages of high and good quality classes, around 57% and 54%, the percentage falls down up to 39% when the fish index is joined. Fish quality indices are sensitive to additional alterations like flow regime disturbances, lack of river

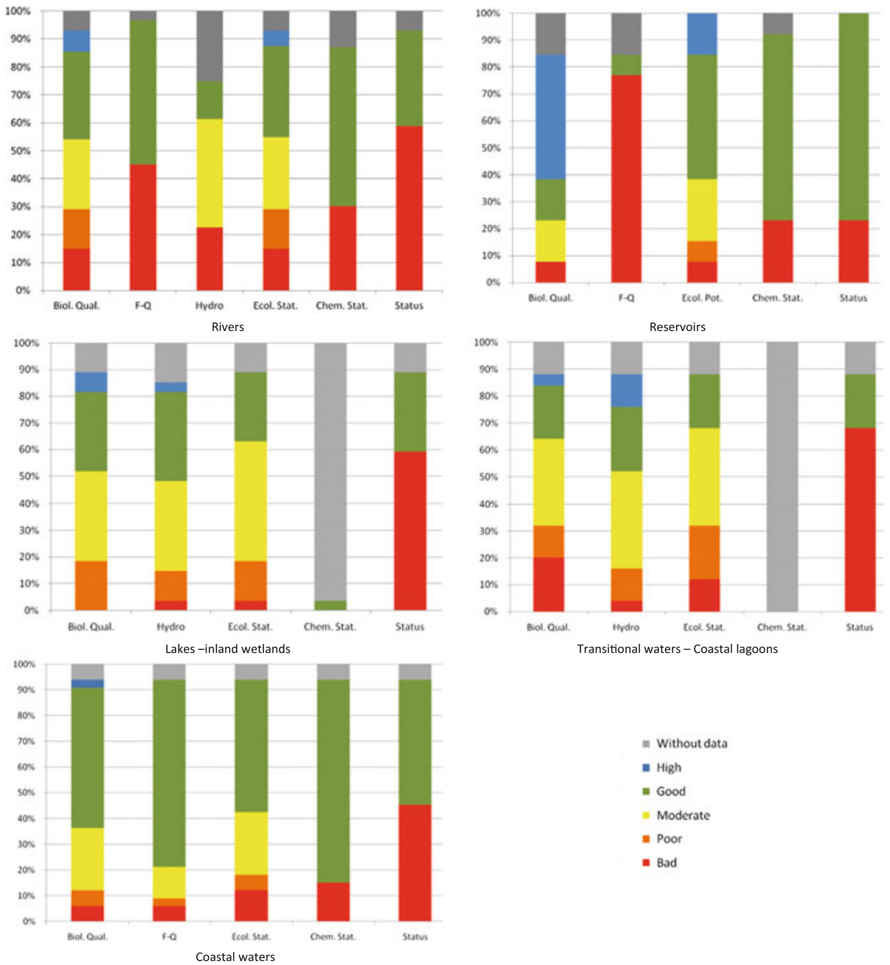

Fig. 2 Outcomes of ecological and chemical status assessed in the Catalan River Basin District. Quality elements are shown for rivers, reservoirs, lakes and wetlands, coastal lagoons (transitional waters), and coastal waters

continuity, or habitat loss [25] in which other biological quality elements are not sensitive enough.

The percentage of streams and rivers classified in good and high quality classes decrease from 39 to 38% when biological quality values are combined together with physicochemical quality values in order to define ecological status. Physicochemical quality shows a 52% of river WB in good quality (128 out of 248), quite similar to 55% which are classified in high and good quality by using macroinvertebrate and diatom biological indices. However, surprisingly a relevant percentage of rivers are not coincident. Some rivers show good biological data but bad physicochemical conditions and vice versa. A total of 14 river WB are classified as high or good

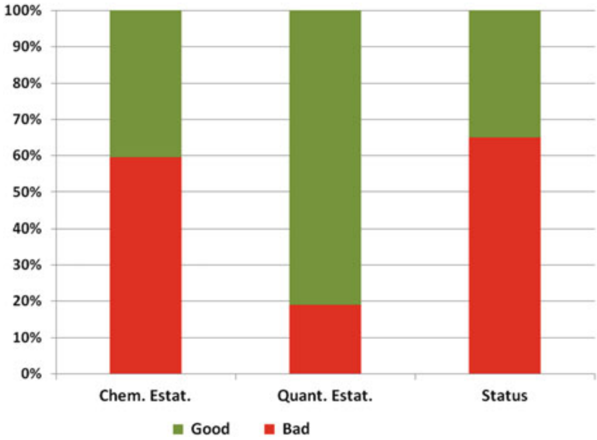

Fig. 3 Outcomes of chemical and quantitative status assessed in the Catalan River Basin District. The final status is also shown by using the one-out all-out criteria

biological conditions but bad physicochemical quality mainly due to high nutrient concentration. Conversely, a total of 24 river WB show moderate to bad biological quality when physicochemical parameters are classified as good. Most of them are affected by hydromorphological alterations, scarce flow regime, or habitat alterations mainly detected through poor fish quality values (low fish density and high alien species). Hydromorphological (hydro) conditions provide additional information to properly interpret biological quality outputs to later set up the final ecological status. Hydro conditions cannot decrease biological quality below good according to the WFD requirements [77]. However, Catalan rivers show a total of 34 rivers with good ecological status (good biological and physicochemical data) but bad hydro conditions mainly due to scarce riparian forest quality and river bed alterations.

Finally, the water body status is established combining ecological and chemical status. We measure chemical status by using quality standards (QS) provided by the Directive 2008/105/EC and 2013/39/EC on priority substances. A total of 141 out of 248 river WB (57%) are classified in good chemical quality. However, combining ecological and chemical status, the number of river WB classified in good status decreases from 95 to 85 (from 38 to 34%). A total of ten rivers with high or good ecological status show bad chemical status mainly located upstream close to non-populated areas. The bad chemical status is mainly due to pesticides (e.g. endosulphan) or heavy metals found in biota (e.g. mercury) at concentrations slightly over the quality standards provided by EU directives.

3.2 *Reservoirs*

Reservoirs are considered heavily modified WB, and their quality status is classified as ecological potential by using metrics shown in Table 6, together with chemical status. Major reservoirs, a total of 9 out of 13 (69%) located in the Catalan River Basin District, are considered in optimum or good biological quality (Fig. 2). Those reservoirs show low concentration of "chlorophyll-a", low percentage of "cyanophytes" in the epilimnion, scarce nutrient concentration, and moderate to high oxygen levels in the hypolimnion. Around a half of reservoirs (7 out of 13) show good physicochemical quality. Finally, two reservoirs are classified as bad chemical status (mainly by heavy metals and pesticides found over the quality standards). Those reservoirs are also classified as bad ecological potential. Reservoirs classified as optimum and good ecological potential are mainly located upstream near natural and non-populated areas.

3.3 Lakes and Transitional Waters

Inland wetlands and coastal lagoons (transitional waters) show quite similar percentage of quality classes. A total of 26 shallow lakes (wetlands) have been defined in the Catalan River Basin District as WB, plus an additional karstic lake (Banyoles lake). Major wetlands and lakes are classified as moderate to bad quality mainly due to flow regime alteration, desiccation, morphological impacts, and chemical contamination from agricultural activities. Only 26% (7 out of 27) of lakes and inland wetlands show god or high ecological status. Physicochemical parameters (nutrients and conductivity) are not used in Catalan wetlands in order to set ecological status because of its high variability over time and to the high values of both in natural conditions [31]. On the other hand, hydromorphological conditions become highly important to diagnose quality status in wetlands and shallow lakes, especially in Mediterranean areas where water is scarce and intensively used by human activities [32], where only 5 out of 14 wetland WB classified as moderate or bad biological quality show good hydromorphological conditions. On the other hand, 3 WB classified as high and good biological quality show poor hydromorphological quality by using ECELS index. Those wetlands are classified as moderate ecological status since morphological alterations heavily affect the aquatic ecosystem but biological indices are not sensitive enough. Chemical status has not been analysed yet due to scarce available data.

Coastal lagoons (transitional waters) show quite similar conditions as inland wetlands. A total of 25 coastal lagoons have been sampled and most of them are highly threatened because they are located close to coastal shoreline where extensive urban areas exist. Most coastal lagoons have been drained and its surface reduced, and only environmental protected areas have been preserved. Thus, only 25% of coastal lagoons show a high or good ecological status (6 out of 25). Chemical status has not been analysed yet due to scarce available data.

3.4 Coastal Waters

A total of 33 coastal WB have been sampled, with several sites for each water body, depending of the natural characteristics of each water body (e.g. type of substratum), which determines the number and the frequency of samples. Moreover, coastal waters are not evaluated by using all biological quality elements in each sampling site or in the same water body. Biological elements are measured only when their potential presence is determined by natural factors. Four biological quality elements are used to define biological quality in the Catalan coastal waters: phytoplankton, macroalgae, seagrass (*Posidonia sp*.), and macroinvertebrates. Biological quality class is obtained by the worst of them. Phytoplankton is measured in 33 WB, whereas macroalgae is measured in sites with a significant proportion of rocky substrate (16 WB), *Posidonia* in 16 WB, and macroinvertebrates in places with sandy substrate (27 WB). Moreover, chemical status is assessed only on water bodies at risk (i.e. in front of urban areas, near large harbours, or around industrial underwater outfalls). Regarding hydromorphological conditions, the methodology has been not developed yet for coastal waters, so they have not been used to define ecological status in coastal waters

Major coastal waters are classified as high or good ecological status (17 out of 33 coastal WB) (Fig. 2). A total of 19 out of 33 coastal waters are classified as high or good biological quality (58%), and 24 out of 33 are classified as good physico-chemical quality (73%). Ecological status is good in almost all northern coastal WB in Catalonia. Main pressures are found in the central coast and south close to large urban areas like Barcelona and Tarragona where the high urban activity affects marine ecosystems. The worst quality occurs close to the metropolitan area of Barcelona and, in front of Tarragona Bay highly influenced by industrial activity, large concentration of population and the presence of river discharges.

The macroinvertebrate quality index (MEDOCC) meets high and good quality values in all WB, whereas phytoplankton shows moderate to bad quality values near urban areas in the north close to Rosas and Figueres cities, in the central coast around the Barcelona area, and in the south near Tarragona city. Macroalgae and seagrass are specially impacted in central and southern Catalan coast, close to Barcelona and Tarragona cities.

Chemical status is evaluated based on priority substances according to the 2008/105/EC and 2013/39/UE Directives. Only WB at risk (in front of the main rivers and major urban and industrial areas) are analysed. Priority substances dissolved in water (measured both coastline and open sea) are very low and thus difficult to detect. However, they are more easily measurable in marine sediments where pollution has been historically accumulated over time. These compounds may come from rivers or urban sewerage systems or industrial sewerage systems. According to the WFD the chemical status of all coastal waters is good.

3.5 Groundwater

Groundwater body status is assessed by combining both chemical and quantitative status using the one-out all-out criteria. Regarding chemical status, monitoring data from surveillance and operational networks are aggregated and compared to TVs and to groundwater QS previously defined according to the Article 3 of GWD. So, bad chemical status is considered: (1) if the area associated with monitoring stations that exceed a relevant chemical parameter is larger than the 20% of the total groundwater body area (using Thiessen polygons to extrapolate values from sites), (2) if the temporal evolution of some relevant parameter significantly increases near TVs, and (3) if local pressures from industrial or agricultural activities show relevant impacts that should be pointed out (i.e. organic compounds or other chemicals as pesticides). Therefore, a total of 15 groundwater bodies out of 37 (41%) were classified as good chemical status, whereas 22 were considered as bad (59%) (Fig. 3). Major impacts were observed for nitrate (NO^{3-}) and chloride (Cl^{-}) which kept 46% and 28% of groundwater bodies below good quality. A total of 17 WB out of 37 show values of nitrate above 50 mg/L, mainly due to intensive farming and fertilization. Those WB are mainly located near agricultural areas or near urban areas. On the other hand, chloride mainly increases in groundwater intensively affected by water abstractions located near shoreline. Thus, water withdrawal causes saline intrusion from seawater and affects water quality in a total of 6 out of 37 groundwater bodies (22%) in the CRBD. Additionally, four non-coastal WB show high chloride values because of industrial activities and mining. Moreover, high values of sulphate (SO^{4-}), ammonium (NH_4^+), perchloroethylene (PCE), and trichloroethylene (TCE) are, respectively, found in 7, 3, 5, and 3 groundwater bodies.

Four elements were taken into account as a criteria for determining the groundwater quantitative status assessment: (1) that the total abstraction from the groundwater body should not exceed the recharge, also considering an allowance for dependent aquatic ecosystems; (2) that groundwater abstraction should not cause a reversal in groundwater flow direction which results in the significant intrusion of saline or other poor quality water into the groundwater body; (3) that groundwater body-related pressures should not diminish groundwater flows supporting terrestrial ecosystems in way such that these ecosystems may suffer "significant damage" in relation to conservation objectives; and (4) that groundwater level monitoring data show an stable tendency over time. Therefore, most groundwater bodies (30 out of 37) show good quantitative status and only 7 bad quantitative status. Most of WB classified as bad quantitative status (6) are also coincident with bad chemical status.

Finally, combining chemical and quantitative status, 13 groundwater bodies were classified as good (35%), whereas 24 were classified as bad (65%) (Fig. 3).

4 Issues to Be Improved on Ecological Status Assessment

Experiences gathered and shared between the Catalan Water Agency (ACA) and several research centres and universities closely involved in the development of the monitoring programme of the Catalan aquatic ecosystems have pointed out some difficulties and inconsistencies to meet certain WFD requirements. Uncertainties in ecological and chemical status assessment may have many sources [60, 78, 79]; we have selected here the most relevant we found applying aquatic ecosystem monitoring in the Catalan basins.

4.1 Hydromorphological Conditions and Ecological Status Relationship

Physicochemical quality of aquatic ecosystems has been enhanced in Catalonia for the last decades due to a large investment on water treatment facilities both for urban and industrial effluents, but pressures on hydrology, ecosystem continuity, and habitat loss have not been appropriately considered. A total of 152 river WB out of 248 (61%) have been classified with poor or bad hydromorphological conditions in the Catalan River Basin District, whereas 112 (45%) have been classified as bad physicochemical quality. Nevertheless, hydromorphological measurements are not required by the WFD to achieve good ecological status, and it is only used to classify WB between high and good ecological status [77]. Thus, ecological status can be set as good while hydromorphological conditions could be poor or bad. Obviously, biological indices are required to be sensitive enough to hydrological pressures, but most biological assessment methods developed so far are to a large extent insensitive to hydrological alterations [25]. In the Catalan River Basin District, some WB have been classified as good ecological quality while hydromorphological quality is poor. In rivers, a total of 34 out of 248 WB (14%) show poor or bad hydromorphological quality but ecological status is classified as good. In wetlands, a total of 4 out of 27 WB (15%) show high or good biological quality but poor hydromorphological conditions, and 3 out of 25 coastal lagoons (12%) also show high or good biological quality but poor hydromorphological conditions. So, hydromorphological quality might be carefully considered when assessing ecological status of a water body subject to an important hydrological alteration, especially when evidence of severe hydrological alterations is not detected through the current biological quality elements.

Benefits of wastewater treatment have been thoroughly documented over the last decades in the Catalan River Basin District [80]. In contrast, the response to hydromorphological restoration has shown to be more complex and less predictable [81]. Thus, we need to better understand and predict the benefits of future river hydromorphological restoration projects and its effects on biological communities in order to improve biological indices which are required to give an integrated quality status assessment [82, 83]. There is an urgent need to gather scientific evidence illustrating how geomorphology supports biota and to improve the understanding of the links between morphology, habitats, riparian forest, and the communities living in the aquatic ecosystems [84]. Many of the existing tools only give a description of condition rather than an understanding of functioning. There is a crucial need to understand the hydromorphological and biological responses to new modifications of water environment and future environmental changes [79, 85].

4.2 The Ecological Status of Temporary Ecosystems

Mediterranean rivers are characterized by frequent natural hydrological disturbances, including floods and droughts. The hydrological regime has become a key element that determines biological community composition and its response to the interannual and seasonal hydrological variability [86]. Numerous studies have revealed the peculiarities of biological communities in Mediterranean and temporary streams (see [13, 87, 88], where annual and interannual changes in the composition of the invertebrate community are found related to flow regime that may fluctuate from perennial to intermittency (presence of permanent pools during the dry periods) until the moment that the channel is totally dry [89]. Thus, biological quality and reference conditions could naturally change between seasons and between years, which made complex the biological quality assessment on such Mediterranean water ecosystems.

Differences between spring (from April to May) and summer (from July to August) samples and dry and wet periods were studied in several Catalan rivers using the macroinvertebrate community assemblages at family level [20]. Four biological quality metrics commonly used in the Catalan basins were compared: IBMWP (Iberian Biological Monitoring Working Party), IASPT (Iberian Average Score Per Taxon), taxon richness at family level, and the number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) at family level (Fig. 4). Differences were large in some of these indexes between dry and wet years than in spring and summer in reference sites. The analysis shown that macroinvertebrate communities and biological indices may significantly change according to hydrological conditions (Fig. 4), clustering the rivers in three different groups: (1) rivers with a continuous flow regime located in siliceous zones, (2) rivers with a continuous flow regime located in calcareous zones, and (3) temporary rivers regardless from the geology. These results agree with other similar works carried out in Mediterranean areas (e.g. [90]) or in other European basins [91]. Temporary rivers appear in all these studies as a heterogeneous group, without a unique typological aggregation but displaying notable dispersion between sites and samples. This phenomenon leads difficult to establish a unified biological quality monitoring and the threshold values for the different metrics between quality classes.

Therefore, biological quality metrics need to be previously analysed in Mediterranean water ecosystems in order to know their temporal or spatial behaviour and

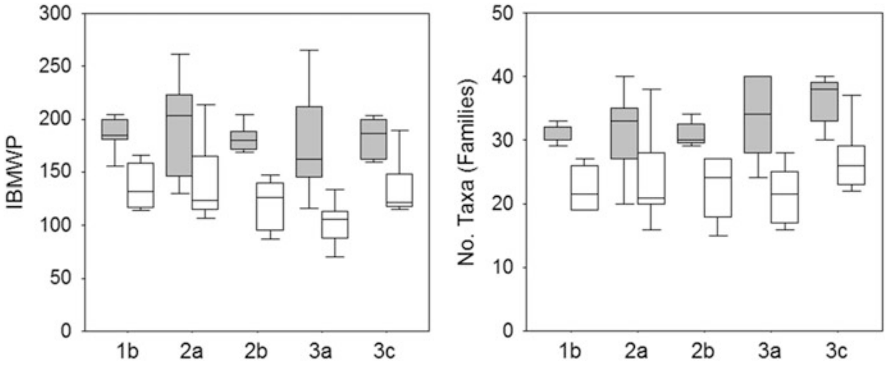

Fig. 4 Results of IBMWP biological quality index and number of taxa of macroinvertebrate (at family level) applied in different river types (1a, 2a, 1b, 3a, and 3b) in the Catalan River Basin District. All values were taken from reference sites (without human pressures and near natural conditions). *Dark box* shows data collected in wet years, whereas *white boxes* show data collected in dry years. Results and more information can be found in Munné and Prat [20]

to select the most suitable biological quality assessment method and reference conditions. For Mediterranean areas, and especially for temporary water ecosystems, the IASPT metric, or some multi-metric indices that use this metric in their formulation, such as the ICM-Star [67], the IMMi-L, and the IMMi-L indices [19], seems to be more appropriate than other ones to establish the biological condition of temporary rivers (Fig. 4). On the other hand, water abstraction and human activity may affect flow disturbances and can change a perennial stream to an intermittent one or increasing the duration and magnitude of droughts and limiting the stream's ability to support aquatic biota [89]. So, what extent the flow regime has been altered through water abstraction or whether scarce or null flow regime is due to natural conditions or human pressures becomes as a key issue to properly assess the ecological status in Mediterranean WB.

Recent approaches to this topic propose to adopt a toolbox including several protocols designed to be used in a sequential manner to allow the establishment of the ecological status of temporary streams and to relate these findings to the hydrological conditions [14, 92]. This toolbox is intended to serve the following purposes: (1) the determination of the hydrological regime of the stream, (2) the design of adequate schedules for biological and chemical sampling according to the aquatic state of the stream, (3) the fulfilment of criteria for designing reference condition stations, (4) the analysis of hydrological modifications of the stream regime (with the definition of the hydrological status), and (5) the development of new methods to measure the ecological status (including structural and functional methods) and chemical status when the stream's hydrological conditions are far from those in permanent streams. The definition of six aquatic states (hyperrheic (floods), eurheic (continuous flow with riffles), oligorheic (connected pools), arheic (disconnected pools), hyporheic (no surface water, alluvium saturated), and edaphic (alluvium not saturated)) by [92] summarized the set of aquatic mesohabitats which occurs on a given stream reach at a particular moment depending on the hydrological conditions and the biological communities to be used to establish the biological quality. Further developments of such approach may be useful to solve the problems found by water managers to establish the ecological status of temporary rivers.

4.3 Chemical and Ecological Status Relationship

Achieving the WB' "good status" as required by the WFD involves fulfilling both the ecological and the chemical good status as well. It seems thus reasonable rising the question of until what extent both quality "dimensions" are interrelated, since the respective results are not always consistent (Table 7). This is a topic of research to which many efforts within the EU-funded research (FP6, FP7, and H2020) as well as studies promoted by member states have been addressed.

In addition to specific topics such as analytical methods development for the different pollutants and matrices at their environmental levels (typically ng/L for many compounds) that is a topic highly correlated with the progress achieved by analytical chemistry [93], here we briefly examine some other key aspects related to more basic issues which remain still open and require further scientific research.

4.3.1 Selection of New Emerging Compounds to Be Included in the Priority List: The Prioritization Process

Good chemical status as defined in the WFD is basically linked to the accomplishment with the "environmental quality standards" (EQS) published for the so-called priority substances. Up to now compounds included in this list are 45 substances. This is in sharp contrast with the fact that more than 100,000 substances are currently in daily use by industry and household, of which ca. 30,000 are of concern and subjected to registration under the new REACH regulation [94]. These highly unbalanced figures simply evidence the limitation of knowledge of the real effects of such substances and the mixtures of them. Even though the WFD foresees

Table 7 Number of sampling sites for each water body category classified as good chemical status but bad (moderate, poor, or bad) ecological status, and vice versa

	Good chemical status and bad ecological status	Good ecological status and bad chemical status
Rivers	69 (28%)	13 (5%)
Reservoirs	3 (23%)	1 (8%)
Lakes and wetlands	–	–
Transitional waters – coastal lagoons	–	–
Coastal waters	10 (28%)	1 (3%)

periodical updates of the list every 6 years, the question is far from being satisfactory solved.

Progress on environmental analytical chemistry has shown the occurrence in the water environment of many not yet regulated substances, globally known as "emerging contaminants": pharmaceuticals, personal care products, illegal drugs, perfluoroalkyl compounds, halogenated flame retardants, endocrine disruptors, pesticides, as well as many industrial compounds have been identified in the environment (together with their transformation products) at non-negligible levels [95]. Furthermore many of them are designed to be bioactive and their long-term exposure effects are largely unknown. Identifying more candidate compounds to be included in the priority list, and what is more important, speeding up the process of inclusion seems thus of key relevance.

4.3.2 Bridging the Gap Between Chemical and Ecological Status: The Ecotoxicity Approach

Ecotoxicity appears as an "in-between" discipline capable to translate chemical exposure into biological effects. Occurrence levels of chemicals can be expressed in terms of risk by comparison to their toxic levels. The toxic unit (TU) or hazard quotient (HQ) (respectively, MEC/EC50 or MEC/PNEC; MEC = measured environmental concentration; PNEC = predicted no effect concentration) approach is commonly used and provides a simple way to quantify the environmental risk associated to a single chemical. In order to be ecologically representative, both TU and HQ should be calculated for different trophic levels (typically, daphnids, algae, and fish) [96]. While the end points considered in ecotoxicological test are not able to encompass the entire ecosystem, it is widely accepted as that ecotoxicology is a reasonable approach to explain until some extent the ecological status, but this approach is not clearly included in the regulations of the WFD.

A further complication arises from the fact that pollutants seldom occur alone; rather they are present in the environment as complex mixtures. Estimation of toxicity of mixtures is currently an issue of active research. Typically two approaches are applied essentially differing on the underlying assumptions as regards the mode of action of the individual constituents composing the mixture. In the so-called concentration addition (CA) [97], substances are supposed to act under a common mechanism, so that their concentrations can be added after weighting them by their respective toxic contribution. Conversely, under the independent action (IA) [98] approach, substances are supposed to act through specific mechanisms. In practice, since mechanisms of action are rarely known for many substances, it is not easy to decide what the best option is, and both approaches should be interpreted as extreme cases defining a "window" in which reality is framed. A further limitation of both approaches is the lack of ability on predicting synergistic or antagonistic effects among the mixture constituents. Owing to the fact that CA model yields higher values (precautionary principle) together with its simplicity of calculation, CA by simple aggregation (i.e. TU or HQ sum of all compounds on a sample) is commonly used as a first-tier approach [99]. Toxic mixture effects are recognized as a key aspect on the interpretation of pollution effects and have been explicitly considered in the last WFD application guidelines [100].

4.3.3 Effects of Hydrology on the Levels of Emerging and Priority Contaminants

Environmental levels of contaminants are typically obtained through monitoring campaigns carried out by the responsible authorities. However, owing to obvious cost of such campaigns in terms of human and analytical effort, they are often limited and the data obtained are generally scarce and unable to cope with their environmental variability caused by both human and natural factors. Among the latter, hydrological factors, such as flow discharge variation, are of key importance and can have a huge influence in the levels and fate of contaminants actually found in the WB. Dilution/concentration effects, increase/decrease of the residence time, resuspension from polluted sediments, effects of turbidity on photolysis, biodegradation, and overflow from WWTPs are just some of the processes that strongly depend on the hydrological conditions. All these effects (and their consequences on the ecosystem) cannot be disregarded particularly in the Mediterranean area, where severe droughts and flash floods can take place within short time intervals. Furthermore, such trend will be accentuated in the future according with the IPCC climate projections [101]. Since, as mentioned above, monitoring cannot be extended unlimitedly in space and time, attention is focused on modelling as an alternative and less costly possibility. While modelling has been extensively developed and applied to hydrology, conservative contaminants, or nutrients, very few attempts have been devoted to emerging microcontaminants. Some promising approaches have been however recently published [102–104].

More generally, the combined effects of different stressors (hydrological, chemical, and related to global change) on the aquatic ecosystems [105] should be explicitly mentioned as key issue deserving further investigation efforts.

5 Discussion and Conclusions

The WFD was welcomed by many for its innovativeness and providing changes towards measuring all surface water status using a range of biological communities rather than only the physicochemical quality or targeted quality for water uses. After several years of scientific work provided from research centres and universities as well as important technical and financial contributions from European member estates and water authorities, a fruitful intercalibration exercise for biological methods was achieved across Europe [69–71], greatly improving homogeneity in the assessment of ecological status even in Mediterranean basins (i.e. [21]).

Likewise, the Catalan Water Agency has introduced new approaches and trend analysis through a close cooperation with research (see Table 1), in order to propose new tools for water body monitoring and water status assessment. Chapters published in both Springer books, "Experiences from Surface Water Quality Monitoring. The EU Water Framework Directive Implementation in the Catalan River Basin District (Part I)" and "Experiences from Groundwater and Coastal Water Quality Monitoring. The EU Water Framework Directive Implementation in the Catalan River Basin District (Part II)", show most of this work. The WFD has been implemented over the last 15 years through continuous work in this area, and clear protocols have been produced for the ecological status assessment. However, the experience gathered so far has make evident some uncertainties on water status classification that should be worth mentioned to be addressed.

One of the main uncertainties detected in the Catalan water ecosystems is focused on intermittent flow regime and droughts. Temporary ecosystems comprise an important water body network in the world, especially in Mediterranean areas, and this proportion is predicted to increase due to global change [106]. The recurrent cessation of water flow influences composition and densities of biotic communities as well as biological quality indices even in reference sites [20, 90]. Several studies focused on the highly adapted biological communities that live in these streams (see [13, 91]). However, they have not been fully integrated into water monitoring so far because most water managers mainly apply perennial river quality assessment principles when making decisions related to temporary ones [14, 15, 92]. The presence of temporary streams in the hydrographical network of drainage basins is a characteristic shared by numerous basins across Europe, not only in Mediterranean areas [91]. Besides, flow regime can be altered due to human pressures, therefore characterizing hydrological conditions prior to the assessment of quality status, and become a key issue to understand and better classify ecological status. Human or natural source of hydrological alterations should previously be assessed. The Catalan Water Agency is currently involved in the LIFE Trivers project especially focused on monitoring and ecological status assessment in temporary rivers (http://www.lifetrivers.eu/en/home). LIFE Trivers studies the hydrology and ecology of temporary rivers and aims at creating new tools to improve their management according to the objectives of the EU Water Framework Directive (WFD), and using the MIRAGE tool box [14], up to 25 WB out of 346 surface waters (7%) have not been analysed in the Catalan River Basin District due to lack of water in the streams when the sites are treated as permanent ones (Table 8). The use of biological quality indices developed over the last 15 years (e.g. IMMi-T quality index [19]) and the new tools designed for hydrological evaluation of intermittent streams should reduce our inability to establish the ecological status of intermittent streams.

Another source of uncertainties arises when chemical and ecological status are combined to assess the water body status or combining all biological quality elements to finally set the ecological status. Elements from the biological calculation are required to be combined considering the CIS guidance documents [77], and therefore the worst quality value from the quality elements used should be adopted

Table 8 Water status, using the four classes defined in this paper, in the Catalan River Basin District for each water body category (data collected from 2007 to 2012)

	Good	Good with uncertainties	Bad with uncertainties	Bad	Without enough data
Rivers	41 (17%)	44 (18%)	81 (33%)	85 (26%)	17 (7%)
Reservoirs	7 (54%)	3 (23%)	1 (8%)	2 (15%)	0 (0%)
Lakes	1 (4%)	7 (26%)	8 (30%)	8 (30%)	3 (11%)
Transitional waters (coastal lagoons)	0 (0%)	5 (20%)	8 (32%)	9 (36%)	3 (12%)
Coastal waters	16 (49%)	0 (0%)	9 (27%)	6 (18%)	2 (6%)
Groundwater	13 (35%)	0 (0%)	0 (0%)	24 (65%)	0 (0%)
All WB	**78 (20%)**	**59 (16%)**	**107 (28%)**	**114 (30%)**	**25 (6%)**

by using the one-out all-out criteria. However, all biological quality values might not have the same weight and the one-out all-out principle can be questioned when biological indices or chemicals provide disparate information from similar pressures. Some WB are classified as bad ecological status when all biological and physicochemical elements are bad, whereas in other cases, WB are also classified as bad status only when one physicochemical parameter is bad, or only few biological values show poor quality. Actually, physicochemical quality of many samples was identified as bad in the Catalan River Basin District, while biological quality is good, and vice versa. On the other hand, also some sites were classified as good ecological status when hydromorphological conditions are poor or bad. It becomes clear that the overall ecological quality assignments are more influenced by physicochemical quality elements than by the hydromorphological elements in the current biological indices. In our opinion, assessment with ecological quality classes computed using the one-out all-out rule when aggregating all the biological quality elements and physicochemical quality (i.e. we picked the worst result as the final ecological status) can provide misleading results and uncertainties. So, a smart analysis is necessary combining quality elements and uncertainties must be solved when classifying water status. For this reason, the ACA is proposing to classify water body status in four categories: "good", "good with uncertainties", "bad with uncertainties", and "bad" (Table 8). "Good status" is set when all quality elements are high or good. Nevertheless, when hydromorphological quality is bad or poor, or when almost all quality elements are high or good, but some chemical or biological elements show some values with poor or bad, then "good with uncertainties" quality class is set. Thus, WB with a clear good status are differentiated from those with unclear or antagonistic results. On the other hand, when all quality elements show bad conditions, water body is classified as "bad". However, in some cases, not all biological elements are bad, or bad conditions have been classified due to some few chemicals or physicochemical results or although the final chemical average shows that bad quality values are improving over time. Then in these cases we propose to use as a category "bad with uncertainties".

Uncertainties or contradictions may lead to some repairs in the way as the ecological status is calculated according to the WFD. The suggested procedure by the CIS in the way of using one-out all-out rules to compute the ecological status (ES) is particularly prone to misclassification when a large number of quality elements are combined in the assessment. Therefore, we have to focus on the ecological meaningfulness of the combined quality elements included in the analysis. In our opinion, the weakest point of the one-out all-out rule when combining all biological and physicochemical quality elements to assess the ecological status is the lack of representativeness and the level of redundancy among them. We acknowledge that assuming the one-out all-out rule may in its turn imply an overly pessimistic and unrealistic result and introduce a bias in the design of program of measures. Therefore, following the uncertainties summarized in this chapter, the establishment of two new quality class categories ("good with uncertainties" and "bad with uncertainties") may be a reasonable solution (Table 8). Results show that a total of 166 WB (43%) have contradictions or uncertainties when the ecological status is adopted in the Catalan River Basin District. A total of 59 of them are finally classified as good (but close to bad) and 107 as bad status (but close to good). The use of such four categories may give a more realistic situation of the status of freshwater ecosystems and produce better fundaments for the establishment of appropriate program of measures.

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A First Biopollution Index Approach and Its Relationship on Biological Quality in Catalan Rivers

Beatriz Rodríguez-Labajos, Carolina Solà, and Antoni Munné

Abstract The purpose of this chapter is to present results of the applicability of the most well-known biopollution (BP) and biocontamination (BC) indices available in the literature by using information from the standard monitoring programme for fish carried out in Catalonia. As a part of this exercise, the pertinence of the results is evaluated by answering two questions: (1) are the BP&BC indices actually indicators for quality status, i.e. do their results respond to indicators of pressures on water bodies? And if so, (2) are the indices redundant with the existing indices of quality status for a given biological element? This discussion will be done in relation to the use of information on alien species (AS) for the purpose of future management and the ensuing role of uncertainty in the ecological assessment on water bodies according to the Water Framework Directive (2000/60/EC).

Keywords Alien species, Biocontamination, Biological indices, Biopollution, Catalan basins, Water Framework Directive

Contents

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Abbreviations

AS	Alien species
BC	Biocontamination
BP	Biopollution
BPL	Biopollution level index
IBPR	Integrated biopollution risk index
SBC	Site-specific biological contamination
WFD	Water Framework Directive

1 Framing the Discussion: Alien Species and the Water Framework Directive

Same than in other regions of its European context, in the Catalan River Basin District, the recognition of alien species (AS) as a pressure to good ecological status has led authorities in charge of implementing the Water Framework Directive (WFD) to develop ad hoc programmes of measures. However, one of the challenges of integrating AS in the management of the ecological status of water bodies is that AS are at the same time a pressure to ecological status and a component of a the biological elements assessed to evaluate ecological status [1]. An enquiry to review how EU Member States deal with AS in their national status assessments unveiled a wide range of practices [2, 3]. This issue was a matter of concern of the WFD Ecological Status Working Group (ECOSTAT) that organised two different technical workshops in 2008 and 2009–with the participation of the lead author–to discuss this topic. In search of a harmonised European approach, ECOSTAT pondered whether AS should be taken into account in the WFD assessment. The starting point was that the Annex V of the WFD states that "water bodies should be 'totally or near totally undisturbed'" in the reference condition. An interpretation of this, for instance, is that WFD precludes the presence of AS at high-quality status. From there, it follows a deliberation about how the impacts of AS are captured in the assessment tools for ecological status classification. The use of supplementary biopollution and biocontamination (BP&BC) indices is one among several options that seem to be favoured by the national authorities in charge of implementing the WFD [2].

Table 1 Biopollution and biocontamination indices from the literature

Index	General description	Data requirements
SBC – site-specific biological contamination index [11]	Based on AS richness and abundance	AS richness and relative abundance per assessment unit
IBPR – integrated biopollution risk index [8]	Risk-based approach with reference to the proportion of AS with potential to spread, establish and cause impact	AS richness and relative abundance per assessment unit
		Evidence of AI impact (either on native biodiversity, ecosystem functions, trophic production, human access to natural resources, human, domestic animal and plant health, recreational and aesthetic activities, infrastructure or control costs)
BPL – biopollution level index [10]	Based on the abundance and distribution of the species and their impact on communities, habitats and ecosystem functions	AS relative abundance and distribution within each assessment unit
		Evidence of AS's impact on native species of communities, on habitats and on ecosystem functioning per assessment unit

The use of the term biopollution to discuss the issue of AS is relatively recent, and it has been basically applied to the aquatic environments [4, 5]. Biological pollution is related to the adverse impacts of invasive alien species due to effects on one or more levels of biological organisation: individual (such as internal biological pollution by parasites or pathogens), population (by genetic change, e.g., hybridisation), community (by a structural shift), habitat (by modification of physical-chemical conditions) or/and ecosystem (by alteration of energy and organic material flow) [6]. It conveys the idea that AS disrupt the ecosystem's health and thus impair the ecological quality of the environment [6, 7]. The adverse effects of biopollution may encompass social and economic costs. The most well-known methodologies to assess biopollution are the integrated biopollution risk index (IBPR) [8, 9] and biopollution level index (BPL) [10]. Another related term, also useful for guiding the management response to AS, is biological contamination or biocontamination (BC) that avoids any reference to potential impacts of the species and therefore is not considered equivalent to biopollution (BP). Biocontamination can be estimated through the site-specific biological contamination (SBC) index [11]. It is worth saying that the normal status classification usually relies on the match between the quality classes and differentiated effects of stressors, which would be a good property to maintain in the integration of AS to the assessment [1, 3]. In the final recommendations of the workshops organised by ECOSTAT, the critical importance of methods for identifying risk and the need to test biopollution indices across all types of surface waters is pointed out, including their application to the procedures of the WFD [12].

Given the relevance of this discussion, the purpose of this chapter is to present results of the applicability of the most well-known BP&BC indices available in the literature (Table 1) using information from the standard monitoring programme in Catalonia (NE Spain). As a part of this exercise, the pertinence of the results is evaluated by answering two questions: (1) Are the BP&BC indices actually indicators of quality status, i.e. do their results respond to indicators of pressures on water bodies? And if so, (2) are the indices redundant with the existing indicators of state for a given biological element? Note that this discussion will be done in relation to the possible use of information on AS for future management and the ensuing role of uncertainty in the assessment on water bodies according to the Water Framework Directive.

2 Applicability of Biopollution and Biocontamination Indices in Catalan Rivers, a Test Using Fish Species

The study area for the test includes 23 watersheds bounded by the administrative limits of Catalonia, with a total area around 32,000 km^2 (NE Spain). As the region features Mediterranean climate, half of the watersheds comprise ephemeral streams. The dataset includes information from sampling sites along the different river typologies present in the study area, occasionally some of the water bodies containing more than one site (Table 2). Environmental and fish community data were available from sites sampled in 2002–2003 ($n_{s2003} = 333$) and 2007–2008 ($n_{s2008} = 311$) as a part of the routine monitoring programme run by the watershed authority, the Catalan Water Agency [13, 14]. In the case of fish, the BIORI protocol secures obtaining the parameters needed for the estimation of the indices SBC and IBPR, namely, AS richness and relative abundance per assessment unit. In particular, abundance is registered both in terms of density (individuals/ha) and in terms of biomass (kg/ha) [13]. It is worth noting that there is absence of fish in 19.5% (in 2002–2003) and 24% (in 2007–2008) of the monitored sites due to diverse circumstances. Examining the data for the period 2002–2003, whereas 2% were sites with a dry river bed – i.e. ephemeral streams without fish according to historical data – or offered bad conditions for fishing (2%), there is a remarkable 15% of sites were the absence of catches indicates adverse conditions for the survival of the fish fauna, clearly in relation to ecological quality issues.

Focussing on the sites with available information about the fish community, and once contrasted the datasets of both monitoring periods, this section analyses BP&BC indices in water bodies in 2002–2003 ($n_{WB2003} = 182$) and 2007–2008 ($n_{WB2008} = 235$). Comparisons are done intersecting available information in coincident water bodies. The assessment of biopollution requires the characterisation of the species according to their native or alien status. This information was obtained from ACA [13] and Sostoa et al. [14] and adapted through expert assessment for the case of *Salmo trutta*, *Anguilla anguilla* and *Phoxinus phoxinus*.

Table 2 Number of sampling sites across different conditions for the two different analysed periods (2002–2003 and 2007–2008)

Number of items	2002–2003	2007–2008
Total sites (n_S)	333	311
Sites without catches (i.e. no fish, dry river bed or bad conditions for fishing)	65	76
Sites with fish catches	268	235
Water bodies with fish catches (n_{WB})	182	235

2.1 *Site-Specific Biological Contamination Index (SBC), a Reasonable Quick Assessment of State*

The site-specific biological contamination index (SBC) enables the comparison of different aquatic ecosystems according to their level of pollution from new taxa, taking into consideration their relative abundance in the ecosystem [11]. Accounting for the proportion of alien taxonomic orders in the community and the relative abundance of alien individuals, the biocontamination can be classified in five levels from 'no' biocontamination (SBC = 0) to 'severe' biocontamination (SBC = 5) and can be inversely interpreted as a contribution from the 'very good' status to the 'very bad' status of the aquatic ecosystem. The levels are determined through different thresholds in the proportion of species richness and/or the alien species abundance (see Fig. 1).

The initial testing done by the developers of this methodology for rivers of Central Europe used macroinvertebrate data compiled from different sources. After that, the SBC index was applied for the case of the Isle of Man, for macroinvertebrate data [15]. In this case, the data consistently relied on the UK Environment Agency guidelines for monitoring sampling, similar to a well-known assessment system for ecological quality of rivers using macroinvertebrates. A similar exercise was undertaken by Šidagyte et al. [16] for the case of invertebrates in Lithuanian lakes. The two latter studies have the explicit objective of analysing the biocontamination results in relation to metrics of ecological status and/or to environmental stressors parameters. While in the first one there was a significant negative relationship between biological quality indices and the SBC indices, in the second case SBC indices were unrelated either to biological quality indices or to stressor variables.

The SBC is not a risk index, since it does not point to possible negative outcomes but to actual adverse ecological consequences that percolate from the presence and abundance of AS. Once the data for the selected taxa is available, the calculation for a given assessment unit is relatively straightforward, though laborious. In Catalonia, the routine monitoring programme for fish offers the possibility of determining the SBC index using indicators of abundance both in terms of the density (number of individuals per hectare) and in terms of biomass (kilograms of alien fish per river hectare).

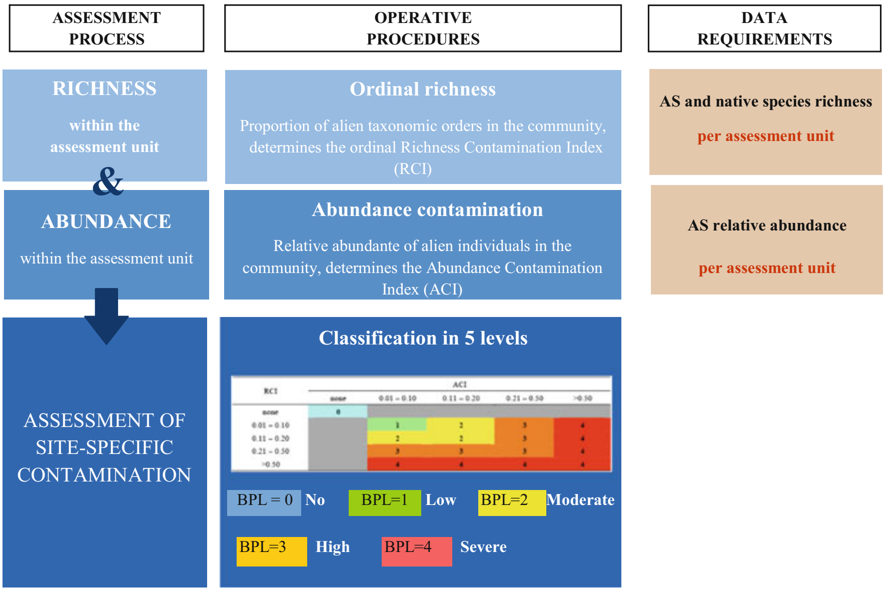


Fig. 1 Procedure for the determination of the site-specific biocontamination (SBC) level. *Source*: Own elaboration based on [11]

The results of the calculation (plotted in Annex I) for the two assessment periods and the two possible metrics of abundance do not differ markedly depending on the metric used (density or biomass). Accordingly there is moderate and more than moderate biocontamination (suggesting less than good ecological status) in one third of the monitored sites (34% in 2002–2003; 35% in 2007–2008) and around a half of the sites with fish communities (47% in both campaigns). In both assessment periods, the results show a negligible worsening (involving up to six sites) when biomass indicators are used, with minor decreases in moderate and high biocontamination and ensuing increases in high and severe biocontamination.

An issue in relation to the use of this indicator is getting polarised results. Most of the resulting biocontamination levels are concentrated at the extremes, as shown in Table 3. Moreover, the presumption of alien species effects simply derived from the alien to native species ratios can be arguable as not all alien species are damaging. In any case, the SBC is an easy-to-estimate indicator based on the existing monitoring routines. It can be used for a quick assessment of the state of biocontamination, provided that there is available data on relative AS abundance at the site level.

Table 3 Percentage of assessment units per SBC level (2007–2008, abundance as kg/ha)

SBC index value	Biocontamination	Number of water bodies (*N*.)
0	No	125
1	Low	0
2	Moderate	2
3	High	40
4	Severe	68
n.a	Without fish	76

Source: Estimated based on data provided by ACA

2.2 Integrated Biopollution Risk (IBPR) Index, a Quick Risk Assessment

Relying on the assumption that risk-based assessments are useful to support cost-effective decisions consistent with the precautionary principle, Panov et al. [8, 9] developed an approach based on the general appraisal of invasiveness according to three elements of risk. Such elements are dispersal, establishment in new environments and generation of ecological and/or socioeconomic impacts, combined as shown in Fig. 2.

The authors also provide some practical guidelines for the evaluation of each one of the descriptors of risk (also indicated in Fig. 2), which involves information about richness and relative abundance of AS in each one of the assessment units. Eventually the IBPR index, scoring from 0 to 4, is estimated with reference to the proportion of species present in specific locations that are included in one or more of three lists (black, grey or white), classified according to a formal listing procedure.

The assessment does not require proof of actual impact in the assessment unit but is entirely based on the existing information about the species' impacts according to the literature or other reliable source of knowledge. Of course, there are different methods to establish generic impact of species. Nentwig et al. [17] propose a scoring system (0–5) using subcategories of environmental and economic impacts multiplying the total rating by the percentage of occupied area and test it for alien mammals in Europe. Magee et al. [18] estimate the magnitude of the stress caused by in situ alien species using an index that summarises the frequency of occurrence and the potential ecological impact, demonstrating the use in the case of streamside vegetation of a river basin. Sandvik et al. [19] classify species based on two axes (invasion potential and local ecological effect), using a list of specific criteria, such as mean expansion rate and interactions with keystone species. They test the proposed system for several AIS still absent from Norway, their geographic area of interest. In the case of the IBPR assessment process, the evaluation is rather simple and only requires one positive response to a list of question about possible types of ecological and socioeconomic impacts (see Fig. 2).

The idea of using standardised procedures to classify AS into grey, white and black lists in order to provide a common framework for management is not new

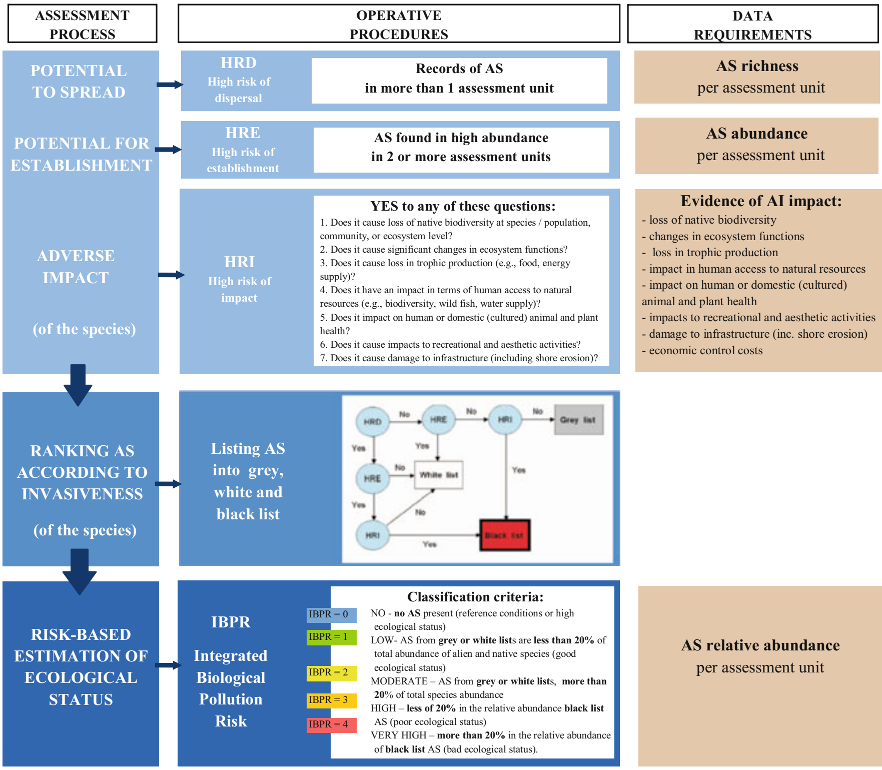


Fig. 2 Procedure for the determination of the integrated biopollution risk (IBPR) index. *Source*: Own elaboration based on [8]

[20, 21]. The IBPR index builds on this background to propose a listing system involving the following categories:

(a) Black list, for species with high potential to cause impact, together with species that are with high potential to spread and establish; their presence should be prevented or deemed as an element of necessary control.
(b) White list, for species with high potential to spread and/or high potential for establishment but low potential to cause impact; their presence can be deemed as acceptable.
(c) Grey list, for species with unknown potential to spread, establish and cause impact; for precautionary reasons, the set of 'no' responses is not interpreted as low-risk potential for all risk elements, but as a need of permanent monitoring to expand knowledge about the species.

Using the information about the number of sites with the presence of the species, their relative abundance and known impacts from the literature, a classification of alien fish detected in Catalonia through the standard monitoring system is presented in Table 4. Note that, in the listing scheme presented in Table 2, 'yes' means that

Table 4 Results of listing species according to the IBPR methodology

Species	HRD (2003)	HRE (2003)		HRD (2007)	HRE (2007)		HRI	List (2003)		List (2007)	
		Ind/ha	Kg/ha		Ind/ha	Kg/ha		Ind/ha	Kg/ha	Ind/ha	Kg/ha
Alburnus alburnus	22	10	3	28	11	6	YES	Black	Black	Black	Black
Ameiurus melas	1	1	1	3	0	0	YES	Black	Black	White	White
Barbatula barbatula	5	2	0	9	4	0	NO	White	White	White	White
Barbus graellsii	26	13	13	25	5	9	NO	White	White	White	White
Carassius auratus	5	2	2	6	3	2	YES	Black	Black	Black	Black
Cyprinus carpio	62	21	39	57	13	32	YES	Black	Black	Black	Black
Esox lucius	1	1	1	1	0	0	YES	Black	Black	Black	Black
Gambusia holbrooki	11	9	1	16	11	2	YES	Black	White	Black	Black
Gobio lozanoi	5	1	0	11	5	1	NO	White	White	White	White
Lepomis gibbosus	18	5	1	23	10	5	YES	Black	White	White	White
Micropterus salmoides	5	1	0	6	0	0	YES	White	White	White	White
Misgurnus anguillicaudatus	N.A.	N.A.	N.A.	1	0	0	YES	N.A.	N.A.	Black	Black
Oncorhynchus mykiss	6	1	4	6	2	4	YES	White	Black	Black	Black
Parachondrostoma miegii	4	4	3	5	3	3	NO	White	White	White	White
Perca fluviatilis	N.A.	N.A.	N.A.	1	0	0	YES	N.A.	N.A.	Black	Black
Phoxinus sp.	18	13	2	31	23	13	NO	White	White	White	White
Pseudorasbora parva	1	0	0	7	0	0	YES	Black	Black	White	White
Rutilus rutilus	2	1	1	14	4	4	YES	White	White	Black	Black
Salmo trutta	9	4	6	3	1	1	YES	Black	Black	White	White
Sander lucioperca	2	0	0	2	0	0	YES	White	White	White	White
Scardinius erythrophthalmus	17	3	2	8	1	1	YES	Black	Black	White	White
Silurus glanis	5	0	1	6	1	4	YES	White	White	White	Black

Gray shade means high risk. HRD high risk of dispersal, based on number of sites with presence of the species (>1), HRE high risk of establishment, based on the number of sites with relative abundance (>20%), HRI high risk of adverse ecological and/or socioeconomic impacts
Source: Own elaboration based on data provided by ACA

information on potential invasiveness of the species is available, while 'no' means information is not available or 'unknown'.

Some comments stemming from the results on listing species are the following ones:

- All the species are classified either in the black or the white lists, and none within the grey one. According to the information on richness and abundance of the listed fish in Catalonia, the only species that could have been considered for the grey list are *Ameiurus melas*, *Esox lucius*, *Misgurnus anguillicaudatus*, *Perca fluviatilis* and *Pseudorasbora parva*. In all cases, available information about impacts of these species has put them automatically in the black list.
- In 12 cases (55% of the assessed species), the classification is consistent across periods and metrics of abundance, either in the black list (*Alburnus alburnus*, *Carassius auratus*, *Cyprinus carpio*, *Esox lucius*) or in the white list (*Barbatula barbatula*, *Barbus graellsii*, *Gobio lozanoi*, *Micropterus salmoides*, *Parachondrostoma miegii*, *Phoxinus* sp., *Sander lucioperca*).

  It is worth noticing that the white-list species are either species native to the Ebro basin and other Iberian watersheds translocated into the IBC – with meagre information about impacts – or high-impact AS which are not very abundant in the water bodies where they are present, which suggests low risk of

establishment. Improved knowledge about the impact of the species or future increase in their abundance would result in a change of the classification from white to black.
- In the other cases, the categorisation changes between or within periods. In five cases (23% of the species), the classification changes between periods, for different reasons. Among the several casuistries, it is remarkable the case of *Rutilus rutilus* that increases dramatically in distribution and relative abundance over time, thus becoming a black-list species. In three cases (13.6% of the species), results for the same period vary according to the metric used for assessing the risk of establishment. This is related with species of high-impact potential that may be locally abundant in numbers but which individuals are smaller in size compared with other caught fish of the community (*Gambusia holbrooki*, *Lepomis gibbosus*) or species which size is bigger than other individuals of the community, although may not be as frequently caught (*Oncorhynchus mykiss*, *Silurus glanis*).

Based on these results about the species, and using the classification criteria mentioned in Fig. 2, the IBPR index for each one of the assessed water bodies can be calculated. The results for the two assessment periods (plotted in Annex II) are more distributed among classes than the ones of the BSC index. Yet they are still polarised results, as it is shown in Table 5.

Results differ slightly depending on the metric used (density or biomass). Using biomass indicators of abundance (kg/ha) tends to bring sites graded from the 2 (moderate) and 3 (high) biopollution risk levels to the 1 (low) and 4 (severe) levels, as nearly symmetrical changes in the number of sites can be observed in relation to the assessment done with density indicators of abundance (individuals/ha). This is probably due to the high abundance of small-sized white-list species. In general, the effect is to obtain slightly worse general results when using indicators of abundance based on fish density. Accordingly, there is an indication of moderate and more than moderate biopollution risk (suggesting less than good ecological status) in one third of the monitored water bodies (29–33%) and around 40% of the water bodies with fish communities.

In summary, the IBPR methodology offers a feasible process to assess potential biopollution in different water bodies in Catalonia, based on certain operative assumptions on the impacts of the species. As a risk index, IBPR method is helpful

Table 5 Percentage of assessment units per IBPR level (2007–2008, abundance as kg/ha)

IBRP index value	Biopollution risk	Number of water bodies (*N.*)
0	No	125
1	Low	12
2	Moderate	16
3	High	27
4	Severe	55
N.A.	Without fish	76

Source: Estimated based on data provided by ACA to frame the need for management with an account of possible impacts of AS. The method does not require proof of actual impacts and therefore does not distinguish properly the different effects that the same species may have in different hosting ecosystems. Besides the results for the different assessment units, the process provides with a (non-stable) classification of alien species according to their potential invasiveness, also a useful management tool.

2.3 Biopollution Level Index (BPL), the (Too?) Perfect Assessment of State

If the purpose of assessing biopollution is to understand changes in ecological quality associated with bioinvasions, a precise recognition of the real effects of AS may be more advisable than the appraisal of their possible impacts. In this respect, Olenin et al. [10] proposed a method able to make an explicit account of AS abundance and distribution ranges, together with the actual impact of the AS on native species or communities, habitats or ecosystem functioning, based on scientific evidence. The evaluation procedure, shown in Fig. 3, provides with a classification of water bodies along five levels from ‘no’ biopollution (BPL = 0) to ‘massive’ biopollution (SBC = 5), which can be inversely associated with levels of biological quality according to the classification scheme of the WFD.

Later on, the method was also refined for its implementation to marine waters [6, 7]. A system to facilitate the BPL calculation and information-sharing based on an online platform was designed by Narščius et al. [22]. This method has been applied in several cases, mostly associated with estuarine or coastal areas in the Baltic using macroinvertebrates of phytoplankton [23–25]. A test of the biopollution levels of coastal areas of Catalonia was also undertaken by Ballesteros et al. [26]. The researchers using this method admit that requires substantial research effort, although praise its usefulness for interregional comparisons and the evaluation of effects of individual AS [23].

A priori, the BPL index has excellent properties to grasp the condition of the water bodies regarding biopollution. However, there are difficulties to implement BPL for the case of fish in rivers of Catalonia so far, for the following reasons:

- Lack of detailed information about the species abundance, ranges of distribution and effects of the species within each one of the water bodies. In particular, in the case of fish, the distribution and mobility within the water bodies is poorly studied.
- There is scientific reluctance to assert impact of fish species in situ, due to the high complexity of the aquatic ecosystems and the number of different stressors involved besides the presence of AS themselves.
- From the management point of view, the large amount of effort and resources needed to improve knowledge about local distribution and actual impacts of

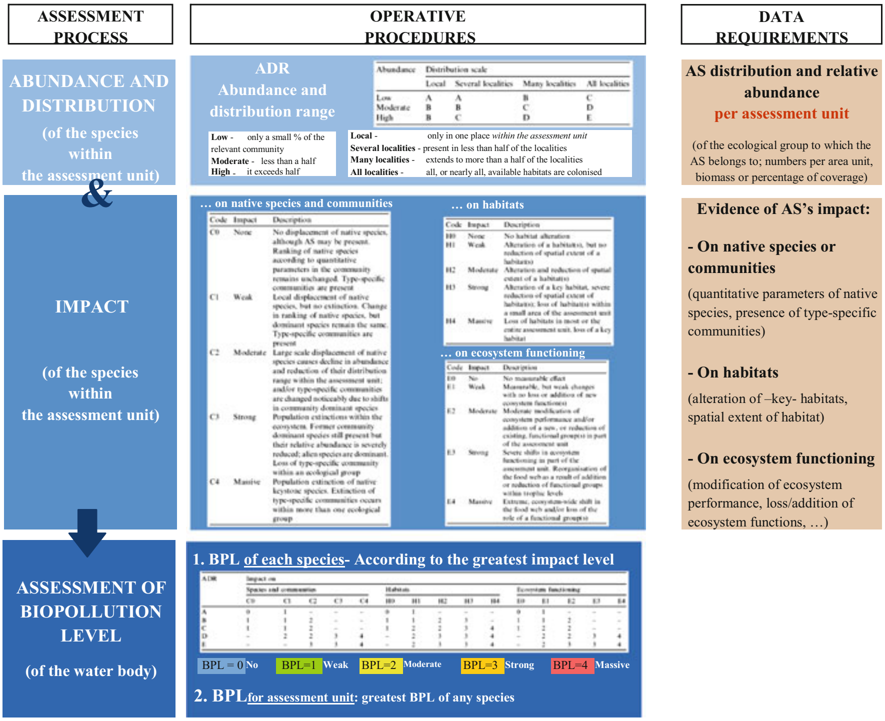


Fig. 3 Procedure for the determination of biopollution level (BPL). *Source*: Own elaboration based on [7].

high-risk AS may be better allocated in preventing the degradation of the state that in confirming *ex post* such degradation.

In sum, in Catalonia BPL could be applied to certain water bodies with the presence of specific AS where research can provide reliable information. That is the case, for instance, of the assessment of biopollution in coastal areas, where the team of researchers in charge have accumulated primary data for decades. In general, that is not the case of fish in river ecosystems, and data requirements for this method largely exceed the current state of data availability. If, in the future, knowledge improves, the BPL is a good candidate indicator for a precise evaluation of the state in relation to biopollution.

2.4 *Comparison of Methods and Use of Results*

To conclude the test of applicability of these methodologies for the assessment of BC&BC, this section elaborates on the use of results and compares the results of the two indices that have been calculated, using the date for 2007–2008, estimated with biomass as indicator of abundance.

There is 82% coincidence in the results between SBC and IBPR. Discrepancies are related to water bodies where there is low abundance of black-list species (with results tending less favourable using IBPR) or areas with high richness of white-list species (with more favourable results using IBPR). In ca. 5.1% of the water bodies, this discrepancy leads to a totally different signal in terms of the assessment, and compliance (in terms of achievement of good status) is dependent on the evaluation method chosen (Table 6).

In relation to the possible use of results, BP&BC can be helpful in several ways. Figure 4, plotting the results of IBPR, will be used as an illustration. First, the

Table 6 Comparison of results SBC and IBPR levels (2007–2008 sampling period) (abundance as biomass). A total of 235 water bodies were analysed

Type or results	SBC values	IBPR values	Number of water bodies	% of water bodies
Same result	0	0	125	53.2
	3	3	18	7.7
	4	4	49	20.9
Different result, same signal	2	3	1	0.4
	3	2	5	2.1
	3	4	6	2.6
	4	2	11	4.7
	4	3	8	3.4
Different signal	2	1	1	0.4
	3	1	11	4.7

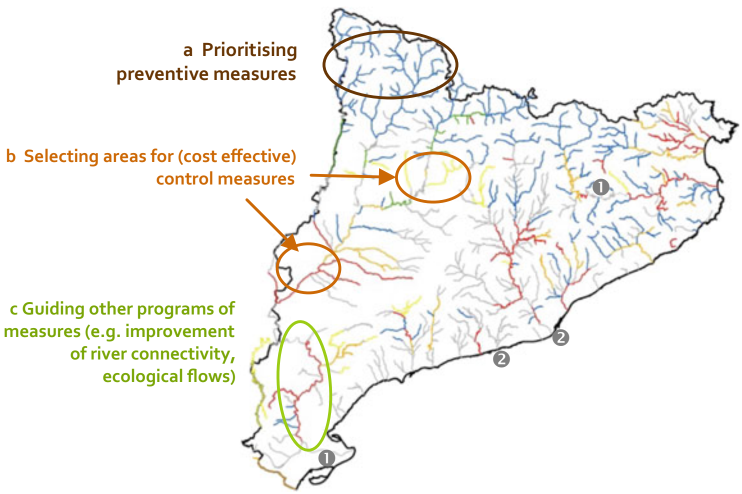


Fig. 4 Results of biopollution risk assessment by using the IBPR index in Catalonia, data gathered in 2007. *Note*: Colours after the corresponding IBPR levels, except for two types of *grey areas*: ❶ water bodies different than rivers; ❷ rivers without fish

identification of areas with low levels of biocontamination or risk of biopollution supports the development of preventive measures, at it is clear that these areas must remain as priority zones for conservation of native species (for instance, the area 'a' in the map). Second, the allocation of available resources can be guided by a cost-effectiveness principle, employing them in areas where the biopollution risk is still moderate or low, instead of where it is severe, and therefore the intervention may result in a future situation of compliance (e.g. the choice between areas 'b' in the map). Third, BP&BC assessment can support programmes of measures with effects in the biotic communities. Thus, for example, the improvement of river connectivity or the implementation of ecological flows, put in place in order to recover the hydromorphological quality of the river, may have also adverse effects in relation to alien species, facilitating their spread to area where they were previously absent. The planning of such measures may take into account likely effects in BP&BC as one of the criteria for intervention.

3 Are the BP&BC Indices Good 'State' Indicators?

As indicated at the beginning of this chapter, a clear association between stressors and the indicator used for quality status is considered a necessary property for the identification of suitable candidates to be state indicators. Then, a pertinent question would be whether the BC&BP levels are correlated with the gradient of pressure in the water bodies.

Anthropogenic activities or actions that may have an impact on ecosystem health are considered to be pressures [27]. In order to characterise the pressures in the sampling sites, the values of a stressor gradient assessment proposed by Munné and Prat [28] for the intercalibration process were obtained. This stressor gradient synthesises the combined effect of different pressures, such as land use types and several types of contamination sources, together with the dilution capacity of the river ecosystem. A general stressor gradient value (that combines quality chemical elements and land use parameters) was available for the year 2003 for water bodies matching 246 sites in with available data on BP&BC in 2003 and 235 sites in 2007.

The scatter plotting of the BP&BC levels and the stressor indicator (Fig. 5) pointed to a certain association of the variables: the higher the stressor value, the highest the BC&BP levels. Some visible outliers were confirmed not to be errors, and therefore they were not excluded from the dataset. Then using a simple bivariate correlation analysis, which indicates how variables or rank orders are related, weak positive linear associations were found between BP&BC and the pressures in the water body. Similar results were found computing the correlations – based on the consideration of BP&BC indicators as ordinal variables – using two nonparametric correlation measures: Spearman's rho and Kendall's tau-b, run with Statistical Package for the Social Sciences (IBM SPSS version 21.0) (Table 7). There is a statistically significant correlation between both SBC and IBPR and the pressure indicators both in 2003 and 2007, with coefficients ranging from 0.215–0.315 (2003) to 0.208–0.313 (2007). For both periods, the IBPR levels were

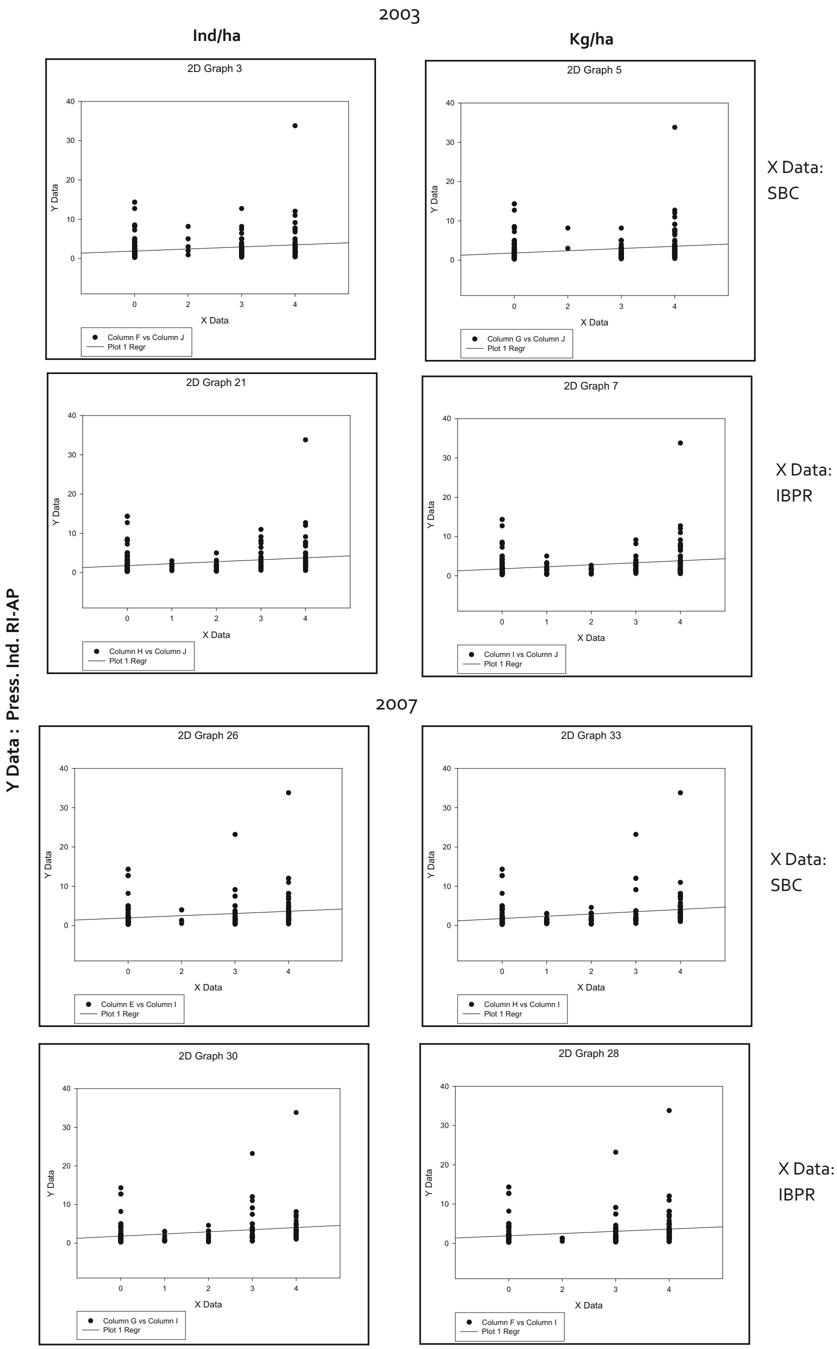


Fig. 5 Scatter plot of stressor gradient across SBC and IBPR levels

Table 7 BP&BC levels and pressures, results of the correlation analysis

Test			RI_AP (BP&BC 2003)	RI_AP (BP&BC 2007)
Kendall's tau-b	SBCindha	Correlation coefficient	0.215**	0.219**
		Sig. (2-tailed)	0.000	0.000
		N	246	235
	SBCkgha	Correlation coefficient	0.240**	0.208**
		Sig. (2-tailed)	0.000	0.000
		N	246	235
	IBPRindha	Correlation coefficient	0.243**	0.224**
		Sig. (2-tailed)	0.000	0.000
		N	246	235
	IBPRkgha	Correlation coefficient	0.240**	0.239**
		Sig. (2-tailed)	0.000	0.000
		N	246	235
	RIAP	Correlation coefficient	1.000	1.000
		Sig. (2-tailed)	–	–
		N	295	311
Spearman's rho	SBCindha	Correlation coefficient	0.274**	0.282**
		Sig. (2-tailed)	0.000	0.000
		N	246	235
	SBCkgha	Correlation coefficient	0.305**	0.271**
		Sig. (2-tailed)	0.000	0.000
		N	246	235
	IBPRindha	Correlation coefficient	0.315**	0.296**
		Sig. (2-tailed)	0.000	0.000
		N	246	235
	IBPRkgha	Correlation coefficient	0.310**	0.313**
		Sig. (2-tailed)	0.000	0.000
		N	246	235
	RIAP	Correlation coefficient	1.000	1.000
		Sig. (2-tailed)	–	–
		N	295	311

Note: **Correlation is significant at the 0.01 level (2-tailed)
Source: Own elaboration. Full results in Annex III

slightly more correlated with the pressure indicator RI_AP than the SBC levels, regardless the indicator of fish abundance used (density or biomass).

The use of the Pearson correlation coefficient was also tested, and it pointed out the same result, although the results are not included in the dissertation as this coefficient is admittedly more appropriate for scale variables.

These results suggest that biopollution and biocontamination are indeed associated with the gradient of pressures to the water bodies, although the current data availability does not point to a very strong association. The knowledge on pressures is expected to improve over time. In case a more precise or sensitive indicator of pressures pointed out to similar or more intense association, the result herein presented would be confirmed.

4 Are the Results of BP&BC Redundant with the Indicators of Biological Quality?

After the WFD, the biological quality of rivers is assessed according to different biological quality elements (BQE): aquatic flora, invertebrates and fish. In relation to the other BQE, fish tend to signal larger spatial and temporal scale processes. As fish are often at the top of the trophic chain, they are sensitive to influences in the rest of aquatic communities. Moreover, fish have relatively higher social visibility and economic relevance than other BQE [13]. Being a part of popular culture and traditional ecological knowledge [29], changes in fish communities can be traced through historical and ethnographic research.

All the above reasons make fish a good base for assessing biological quality. Among the different methodologies developed in this respect, the indices of biotic integrity based on Karr [30, 31] have become widely accepted. This conceptual approach assesses the composition and diversity of species, their abundance and the conditions of the fish. In Catalonia, the index based on this approach, first developed in 2003 [32] and further refined in 2010 [14], is called IBICAT. It was commissioned by the watershed authority that uses it for guiding water quality assessment in rivers, together with indicators for the other BQE [13]. The index allows generating different quality levels based on the score for the different metrics included.

The process to refine IBICAT took particular care of the issue of alien species during the stage of selecting candidate metrics to be part of the assessment. Then, it is a pertinent question whether the results of this index in relation to the issue of AS made it redundant the calculation of an ad hoc BP&PC indicator as the ones that have been tested in this section.

In order to compare both types of information, data on the scores (from 1 to 5) for two different versions of the index ($IBICAT_{2010}$ [$n_{WB} = 234$], $IBICAT_{2b}$ [$n_{WB} = 235$]) was obtained, with permission of the watershed authority, for rivers in Catalonia. The data corresponds to the fish monitoring in the period 2007–2008, that is, the same raw data that they used for the calculation of the BP&BC indices of that period. Levels 1 and 2 correspond to very good and good quality level and, therefore, would point at water bodies in compliance with the WDF; levels 3, 4 and 5 correspond to moderate, deficient and bad quality levels and would indicate incompliance with the WFD.

The results of the different quality levels for the both versions of the IBICAT index, compared with the corresponding level of BP&BC, are shown in Table 8. The cells highlighted in light brown indicate the water bodies in which the assessment of biological integrity and BP&BC provide the same signal (either compliance or incompliance). Meanwhile, white cells indicate divergent results between these two kinds of assessment.

Based on this table of frequencies, it is possible to calculate the probability of coincident results and non-coincident result, shown in Table 9. Looking at the different combinations of indices, it is clear that the probability of coincident results (ranging between 79% and 88%) is always higher than the probability of non-coincident results (12–21%). Being the probability of coincident results remarkably in both versions of the biological quality index, IBICAT_{2b} seems to capture better the issue of BP&BC than IBICAT_{2010} for each one of the indices and metrics used for the assessment of BP&BC.

Focussing on the non-coincident results, two situations are possible: that BP&BC indices indicate compliance, while the biological quality index indicates incompliance, or the other way around. The first situation may be explained by the fact that the fish community suffers from a pressure unrelated to the issue of alien species. The second situation is more problematic from the point of view of the topic addressed in this dissertation. If the biological quality index indicates compliance, there would not be any signal for the water managers to engage in policy measures of ecological improvement, as the state of the water body would be

Table 8 Crosstabs of BC&BP levels and scores of the biological quality assessment for fish, frequencies, $n_{\text{WB}} = 234, 235$, sampling period 2007–2008

BC&BP level		**IBICAT_{2010} Score**					**IBICAT_{2b} Score**				
		1	2	3	4	5	1	2	3	4	5
SBC(ind/ha)	0	30	60	17	13	5	29	77	18	1	0
	2	0	1	1	1	0	0	1	0	2	0
	3	1	9	21	8	0	2	4	22	11	0
	4	0	1	13	34	19	1	2	11	40	14
SBC(Kg/ha)	0	30	60	17	13	5	29	77	18	1	0
	2	0	1	0	1	0	0	1	0	1	0
	3	1	8	23	8	0	3	4	22	11	0
	4	0	2	12	34	19	0	2	11	41	14
IBPR(ind/ha)	0	30	60	17	13	5	29	77	18	1	0
	1	0	3	4	0	0	1	2	4	0	0
	2	0	3	5	11	0	2	2	8	6	2
	3	1	4	16	9	3	0	2	16	15	0
	4	0	1	10	23	16	0	1	5	32	12
IBPR(kg/ha)	0	30	60	17	13	5	29	77	18	1	0
	1	0	5	6	1	0	3	2	5	2	0
	2	0	2	3	10	0	0	2	7	5	2
	3	1	3	13	9	1	0	1	13	13	0
	4	0	1	13	23	18	0	2	8	33	12

Table 9 Coincidence of results between BC&BP levels and biological quality scores. Probability of coincident/non-coincident results

BC&BP level		IBICAT2010 Score 1 2	IBICAT2010 Score 3 4 5	IBICAT2b Score 1 2	IBICAT2b Score 3 4 5
SBC(ind/ha)	0 2 3 4	20 %	80 %	12 %	88 %
SBC(Kg/ha)	0 2 3 4	20 %	80 %	12 %	88 %
IBPR(ind/ha)	0 1 2 3 4	21 %	79 %	13%	87%
IBPR(kg/ha)	0 1 2 3 4	21%	79%	13%	87%

Note: Compliance (C) means levels 0,1 for BC&BP and scores 1,2 for biotic integrity indicators; noncompliance (NC) means levels 2,3,4 for BC&BP and scores 3,4,5 for biotic integrity indicators

considered as good or very good from the point of view of the fish communities. However, the BP&BC indices would be pointing out at the existence of a problem of bioinvasions in that particular water body.

With this in mind, the conditional probability of these two situations was estimated for the different indicators involved (Table 10). In conditional probabilities, they are calculated according to the formula $P(A|B) = P(A \cap B)/P(B)$ when $P(B) > 0$, where the event of interest A is either the biological quality indicator (BQI)'s noncompliance (NC) or compliance (C) and the restricted sample space B is the opposite result in BC&BP level. The results shown in the table indicate that the probability of BC&BP compliance and biotic integrity incompliance (highlighted in orange) ranges between 15% and 30%, and it is always higher than probability of BC&BP incompliance and biotic integrity compliance (highlighted in purple), ranging between 4% and 12%.

This later result is relevant, because it demonstrates that the standard quality assessment fails to completely pinpoint the issue of alien species. While the probability that this happens is relatively low, the failure is systematic regardless the indicator used. Of course, the considerations on uncertainty about the BP&BC indices presented along this section should be taken into account when interpreting this result.

In any case, based on the results presented in this section, it can be argued that the biological quality index used for fish in Catalonia and the BP&BC indices are not redundant. While there is an undeniably high level of coincidence between their

Table 10 Coincidence of results between BC&BP levels and biological quality scores. Conditional probability of non-coincident results

BC&BP level		IBICAT$_{2010}$ score		IBICAT$_{2b}$ score	
		C	NC	C	NC
SBC (ind/ha)	C		28%		15%
	NC	12%		9%	
SBC (kg/ha)	C		28%		15%
	NC	12%		9%	
IBPR (ind/ha)	C		30%		17%
	NC	9%		6%	
IBPR (kg/ha)	C		30%		19%
	NC	7%		4%	

Note: Compliance (C) means levels 0,1 for BC&BP and scores 1,2 for biotic integrity indicators; noncompliance (NC) means levels 2,3,4 for BC&BP and scores 3,4,5 for biotic integrity indicators

results, they do not reflect the same thing, and there is a small probability of systematic failure of the BQI to provide the required policy signals.

5 Concluding Remarks

The consideration of AS in the assessment of biological quality is necessary whenever there is evidence that AS constitute a pressure to or have an impact on the aquatic ecosystem. Some voices even claim that the high ecological status is unsuited for water bodies where AS are present. Yet taking up AS until the last consequences in ecological status assessment may be problematical for water managers. In Catalonia there are practically no water bodies without alien species. The eradication of most of them is environmentally or economically unfeasible. Should a strict AS-based quality assessment be adopted, the water policies would be locked in the predicament of recognising a problem of generalised poor ecological status without being able to effectively redress this situation. In this context, the existence of supplementary BP&BC indices is helpful to guide policies in support of increased biological quality. In the case of Catalonia, and using fish as biological element, two of the methodologies present in the literature can be estimated with the existing monitoring data and would not require further sampling effort beyond the routine monitoring.

The BP&BC indices thus estimated undoubtedly provide useful information for the management of AS in aquatic ecosystems. The classification of water bodies or, as a part of the calculation of IBPR, a classification of the AS themselves helps to prioritise efforts, targeting those management units or species whose control will have the most benefit for the available resources. In the case of the species, such a classification could be easily linked to regulatory frames. For instance, it could be helpful to communicate to the general public why the possession, sale or any other kind of management is restricted for ‘black species’. In fact, impacts of the species are explicitly taken into account in two of the methodologies introduced, although in one case the impact is presumed based on the information from the literature and in the other requires actual evaluation in situ. A consideration regarding species' impacts is the extent to which the criteria for classification are discussed with stakeholders. Although the assessment itself must be guided by a systematic organisation of knowledge and, therefore, can be considered as a scientific endeavour, an agreement with stakeholders on the reasons why a particular species is considered as a hazard will benefit both the comprehensiveness of the analysis and the use of its results in policymaking.

In general, the indices fall short of portraying species whose impacts are not completely understood. Additionally, an element that is absent from the different BP&BC indicators, and that it would be likely to emerge as a result of an open discussion about AS impacts and biopollution, is the recognition of the ambivalence of the species. From the ecological point of view, the potential benefits of alien species include providing habitat or food resources to rare species, serving as functional substitutes for extinct *taxa* and providing desirable ecosystem functions [33]. Moreover, many of the AS, as some of those present in Catalonia, are economically important. Despite this, there is such a scant research done on the potential conservation benefits of alien species that make it think that the topic is a scientific taboo. With increase knowledge about these potential benefits, a new challenge would rise on the best way to integrate it in BP&BC assessment: can benefits be an offset for negative effects of the species?

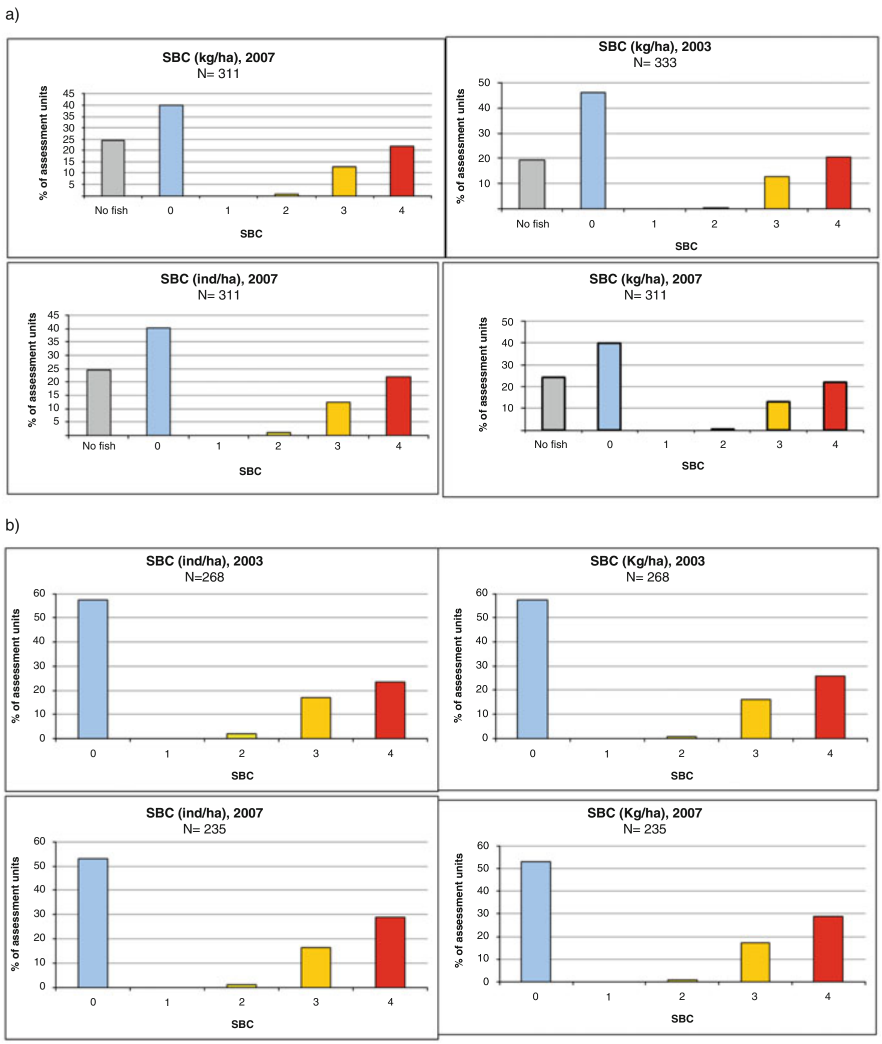
This chapter closes with some final recommendations informed by the testing and analyses done. A major point here is that water bodies are not necessarily homogeneous in terms of the represented habitats, overall all in relation to flora species. A relative abundant species may cause diverse impact depending on the type of habitats along the water body. As a result, the attribution of the impact on habitats may differ. Therefore, a more precise assessment of biopollution, based on actual information about AS impacts, would benefit from changes in the monitoring protocols that involved data gathering about local distribution and effects on local ecosystems and biodiversity, even if it is under qualitative basis.

Another point is referred to the taxonomic groups to be included in the analysis. Due to data availability reasons, the assessment in this section has relied on fish species. As indicated above, most of the tests of biopollution and biocontamination have been done using macroinvertebrates. Potentially, the methodology can be used with any *taxa*. Then a question would be whether other types or organisms with very likely negative effects in ecological status (e.g. zoonotic organisms like parasites) should not be explicitly addressed outside the classical BQE including in the assessment of ecological state.

Acknowledgements The first author benefited from her participation in the formal discussions on alien species and water body classification within for the WFD Ecological Status Working Group (ECOSTAT) in Bordeux (2008) and Ispra (2009), both coordinated by Professor Phil Boon (SNH, UK) and Dr Ana Cristina Cardoso (JRC, Italy). The author gratefully acknowledges the coordinators and all the participants for the fruitful discussions, in particular to Jochen Vandekerkhove (JRC, Italy), for the management of the ECOSTAT questionnaire to national experts 'Alien species and the Water Framework Directive' issued in January 2009.

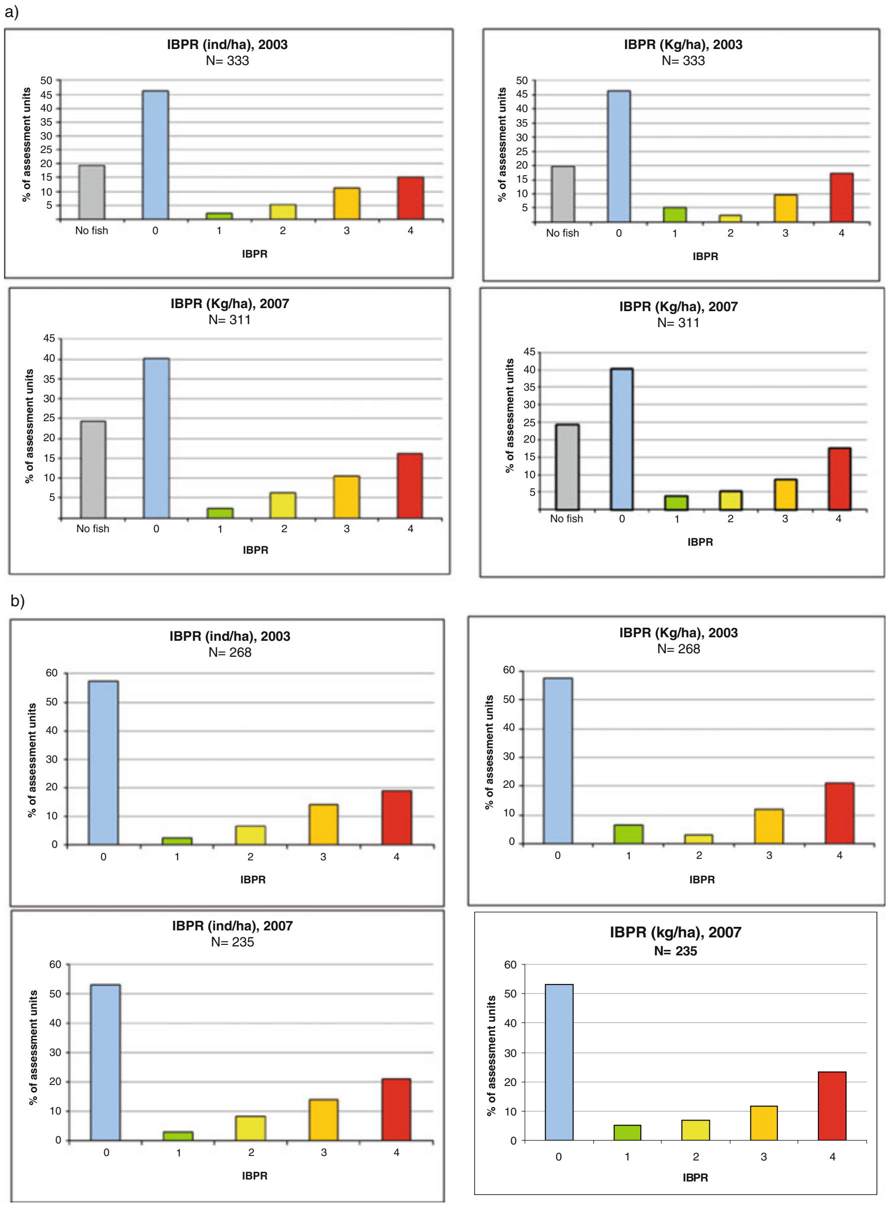
Annexes

Annex I



Results of the determination of the site-specific contamination level, using fish, for the water bodies in Catalonia (2002–2003 and 2007–2008), for different indicators of AS abundance (density [ind/ha] and biomass [kg/ha]). (**a**) Results for all assessment units. (**b**) Results for assessment units with fish fauna

Annex II



Results of the determination of biopollution risk index, using fish, for the water bodies in Catalonia (2002–2003 and 2007–2008), for different indicators of AS abundance (Ind/ha and kg/ha). (**a**) Results for all assessment units. (**b**) Results for assessment units with fish fauna

Annex III

BC&BP levels and pressures, results of the correlation analysis

			SBCindha	SBCkgha	IBPRindha	IBPRkgha	RIAP
Data on BP& BC in 2003							
Kendall's tau-b	SBCindha	Correlation coefficient	1.000	0.924**	0.891**	0.836**	0.215**
		Sig. (2-tailed)	–	0.000	0.000	0.000	0.000
		N	246	246	246	246	246
	SBCkgha	Correlation coefficient	0.924**	1.000	0.899**	0.902**	0.240**
		Sig. (2-tailed)	0.000	–	0.000	0.000	0.000
		N	246	246	246	246	246
	IBPRindha	Correlation coefficient	0.891**	0.899**	1.000	0.907**	0.243**
		Sig. (2-tailed)	0.000	0.000	–	0.000	0.000
		N	246	246	246	246	246
	IBPRkgha	Correlation coefficient	0.836**	0.902**	0.907**	1.000	0.240**
		Sig. (2-tailed)	0.000	0.000	0.000	–	0.000
		N	246	246	246	246	246
	RIAP	Correlation coefficient	0.215**	0.240**	0.243**	0.240**	1.000
		Sig. (2-tailed)	0.000	0.000	0.000	0.000	–
		N	246	246	246	246	295
Spearman's rho	SBCindha	Correlation coefficient	1.000	0.965**	0.954**	0.927**	0.274**
		Sig. (2-tailed)	–	0.000	0.000	0.000	0.000
		N	246	246	246	246	246
	SBCkgha	Correlation coefficient	0.965**	1.000	0.957**	0.959**	0.305**
		Sig. (2-tailed)	0.000	–	0.000	0.000	0.000
		N	246	246	246	246	246
	IBPRindha	Correlation coefficient	0.954**	0.957**	1.000	0.964**	0.315**
		Sig. (2-tailed)	0.000	0.000	–	0.000	0.000
		N	246	246	246	246	246
	IBPRkgha	Correlation coefficient	0.927**	0.959**	0.964**	1.000	0.310**
		Sig. (2-tailed)	0.000	0.000	0.000	–	0.000
		N	246	246	246	246	246
	RIAP	Correlation coefficient	0.274**	0.305**	0.315**	0.310**	1.000
		Sig. (2-tailed)	0.000	0.000	0.000	0.000	–
		N	246	246	246	246	295

(continued)

Data on BP& BC in 2007							
Kendall's tau-b	SBCindha	Correlation coefficient	1.000	0.967**	0.886**	0.877**	0.219**
		Sig. (2-tailed)	–	0.000	0.000	0.000	0.000
		N	235	235	235	235	235
	SBCkgha	Correlation coefficient	0.967**	1.000	0.894**	0.900**	0.208**
		Sig. (2-tailed)	0.000	–	0.000	0.000	0.000
		N	235	235	235	235	235
	IBPRindha	Correlation coefficient	0.886**	0.894**	1.000	0.959**	0.224**
		Sig. (2-tailed)	0.000	0.000	–	0.000	0.000
		N	235	235	235	235	235
	IBPRkgha	Correlation coefficient	0.877**	0.900**	0.959**	1.000	0.239**
		Sig. (2-tailed)	0.000	0.000	0.000	–	0.000
		N	235	235	235	235	235
	RIAP	Correlation coefficient	0.219**	0.208**	0.224**	0.239**	1.000
		Sig. (2-tailed)	0.000	0.000	0.000	0.000	–
		N	235	235	235	235	311
Spearman's rho	SBCindha	Correlation coefficient	1.000	0.983**	0.949**	0.944**	0.282**
		Sig. (2-tailed)	–	0.000	0.000	0.000	0.000
		N	235	235	235	235	235
	SBCkgha	Correlation coefficient	0.983**	1.000	0.953**	0.956**	0.271**
		Sig. (2-tailed)	0.000	–	0.000	0.000	0.000
		N	235	235	235	235	235
	IBPRindha	Correlation coefficient	0.949**	0.953**	1.000	0.985**	0.296**
		Sig. (2-tailed)	0.000	0.000	–	0.000	0.000
		N	235	235	235	235	235
	IBPRkgha	Correlation coefficient	0.944**	0.956**	0.985**	1.000	0.313**
		Sig. (2-tailed)	0.000	0.000	0.000	–	0.000
		N	235	235	235	235	235
	RIAP	Correlation coefficient	0.282**	0.271**	0.296**	0.313**	1.000
		Sig. (2-tailed)	0.000	0.000	0.000	0.000	–
		N	235	235	235	235	311

**Correlation is significant at the 0.01 level (2-tailed)

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The Use of Diatoms to Assess the Ecological Status in Catalan Rivers: Application of the WFD and Lessons Learned from the European Intercalibration Exercise

Elisabet Tornés and Sergi Sabater

Abstract The biological communities have been widely applied in the assessment of the ecological status of water bodies. In particular, diatom communities integrate the environmental effects of water chemistry, along with the physical and geomorphological characteristics of rivers and lakes. The European Water Framework Directive (WFD) included for the first time in Europe the concept of ecological status of aquatic ecosystems in water quality evaluation, based on the use of biological quality elements (BQE) in a type-specific context. During the implementation of the WFD in Catalan rivers using diatoms, 152 stream and river sites were sampled, and the applicability of existing diatom indices to monitor water quality in Catalan rivers was tested. The correspondence between the already proposed typological classifications of rivers and the biological classification was also examined. Since the bioassessment methods using diatoms needed to be comparable amongst different fluvial ecosystems in Europe, several intercalibration (IC) exercises were done throughout Mediterranean areas in Europe. The Mediterranean IC exercise faced the inconsistency between the river types and the biotic classification, the lack of real pristine sites and the existence of taxonomic discrepancies. In spite of these constraints, the Intercalibration Common Metric (ICM) consistently related with the local-used indices (IPS) in all the countries tested. However, during this process, the need of revising the river typology as well as of revisiting the fine-tuning of taxonomic identifications was clear. Putting effort in these aspects would improve the water quality assessment at the national level and would also improve the subsequent comparability amongst countries.

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Keywords Bioassessment, Catalan rivers, Diatoms, Indices, Intercalibration, Mediterranean

Contents

1 The Use of Diatoms as Indicators of the Riverine Ecological Status

Chemical water quality is useful to define the relevance of pollutants in freshwaters but – unless linked to long-term analyses – does not detect changes over a long time scale and does not necessarily reflect the ecological state of the system. Biological elements integrate the environmental factors defining the physical and chemical environment. Bioassessment is therefore an appropriate alternative to purely chemical analyses in rivers and lakes [1]. In the particular case of the microorganisms, their short generation time makes them appropriate early warning indicators of changes occurring in aquatic habitats [2]. Diatoms are part of these microorganisms, and because of their function as primary producers and their dominance in river systems with respect to others [3], they are able to quickly react to environmental changes [4, 5]. Their structural elements in the siliceous cell wall allow reliable taxonomic determination at specific and subspecific level (Fig. 1).

Moreover, diatom sampling requires minimal effort and causes no impact to the sampling site. In addition to easy sampling, diatoms are easily preserved and maintained in permanent microscope slides. After their examination by skilled personnel, diatoms can be used as reliable indicators of pH, salinity, nutrients and even pollutant toxicity. Diatom communities have been proved to be effective biological indicators of aquatic systems in several studies in Europe, and their use and application as indicators of the ecological state of the river is well protocolised (e.g. [6, 7]). Standardised protocols have been elaborated by the European Committee for Standardisation (CEN) for the routine sampling and pretreatment of diatom samples [8] as well as for their identification and counting [9], as a first step to make data amongst different studies and countries being comparable.

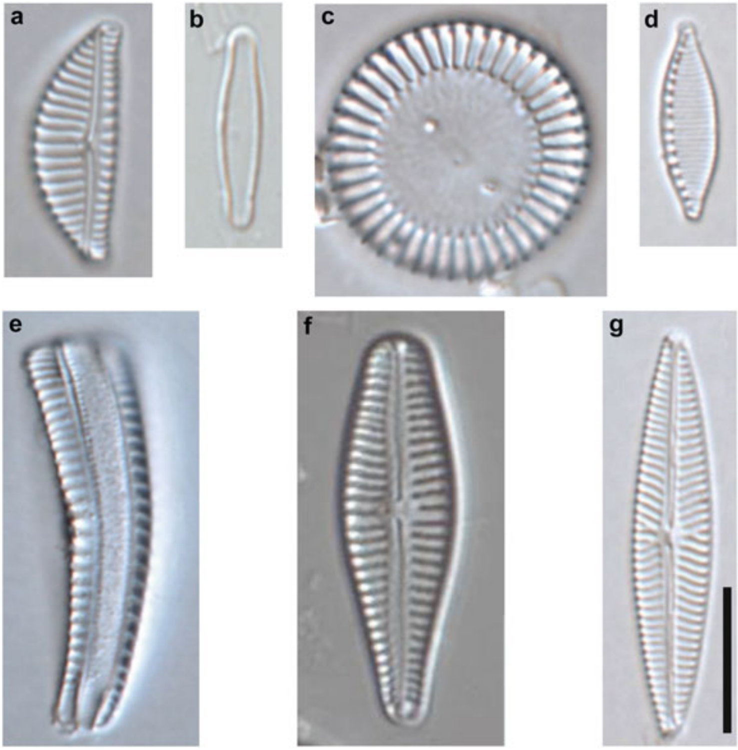


Fig. 1 Diatom species at the light microscopy (1000×) after digestion of the organic matter surrounding the siliceous cell wall. (**a**) *Encyonema silesiacum*, (**b**) *Achnanthidium minutissimum*, (**c**) *Cyclotella meneghiniana*, (**d**) *Nitzschia fonticola*, (**e**) *Rhoicosphenia abbreviata*, (**f**) *Gomphonema clavatum*, (**g**) *Navicula cryptotenella*. Scale bar = 10 μm

2 Implementation of the Use of Diatoms as a BQE in the Catalan Rivers

2.1 Selection of Sites According to the Type of Pressures

The European Water Framework Directive (WFD) [10] included the concept of ecological status of aquatic ecosystems in water quality evaluation for the first time in Europe. This assessment is largely based on the use of biological quality elements (BQE), which includes from algae to macroinvertebrate and fish. Phytobenthos is one of these biological quality elements used in the definition of ecological status, and the taxonomic composition of benthic diatoms has been widely applied as reliable proxies for phytobenthos in Mediterranean freshwaters [11].

Several studies were launched by the Catalan Water Agency (ACA) in order to apply the WFD for the different BQE in the inner Catalan catchments. In the case of diatoms, 152 stream and river sites were sampled during summer (July–August) 2002 and spring (May–June) 2003. Whilst most of these sites (106) coincided with those of the control network of the ACA, other 46 sites were selected to complement the network, mainly in the unexplored headwaters of large catchments (Fig. 2). The selected sites covered a wide range of fluvial typologies, ranging from siliceous high-mountain fluvial systems to coastal streams, and included both calcareous and siliceous Mediterranean fluvial systems, as well as different levels of human disturbance. The smaller rivers (e.g. the Francolí, the Gaià) have their headwaters in middle mountains and flow for a few kilometres to the sea. The larger systems, the Ter and the Llobregat, have their headwaters in the Pyrenees and therefore the upper courses are partially subjected to a snow-fed regime. The Catalan tributaries of the Ebre catchment also have their headwaters in the Pyrenees and experience minimum water temperatures, annual rainfall of above 1,000 mm and heavy snowfall in winter. The middle and lower parts of the Ebre, the Llobregat and the Ter are subjected to a Mediterranean climate, implying high hydrological variability in these sections.

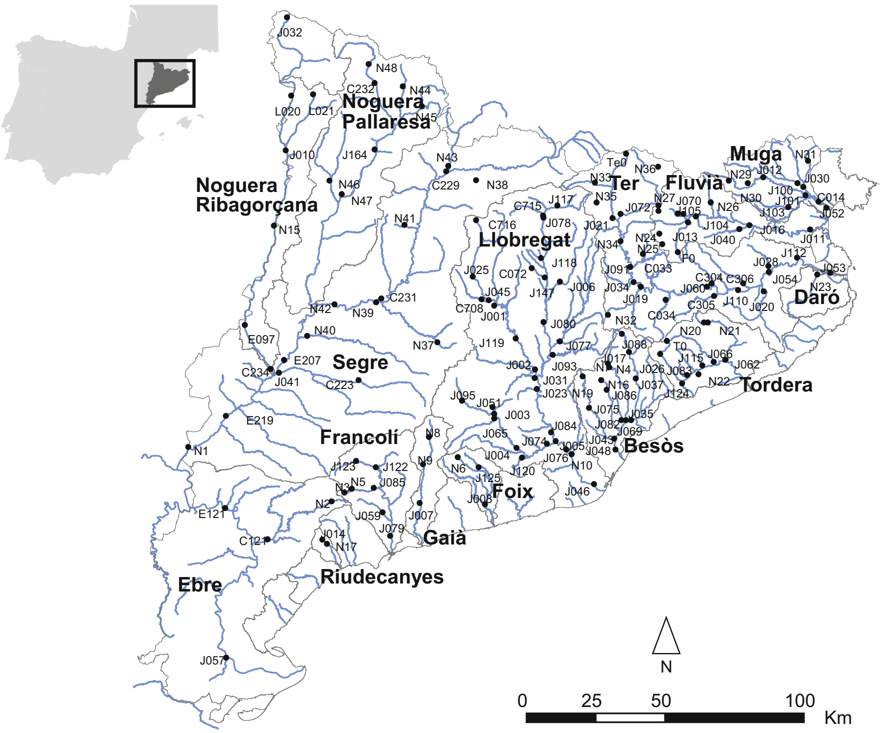


Fig. 2 Map of the area and location of the sampling sites. New sites are indicated with a *N*

2.2 Application and Suitability of Diatom Indices in Catalan Rivers

Diatom indices are designed to summarise the information provided by the autoecological preferences of the diatom community [2]. Several indices have been created and tested mainly in central and northern European rivers, and their applicability to monitor water quality was assessed in the selected sites. The diatom indices tested were the IPS (Indice de Polluosensibilité Spécifique [12]), the IBD (Indice Biologique Diatomées [13]) and the CEE [14]. They were selected for their wide application (IPS and IBD) and for their interest to approach a standard for most European situations (CEE). The indices take into consideration the structure of the community and therefore consider not only the taxa presence but also their proportion in the community. These indices were created by adapting the formula designed by Zelinka and Marvan [15], which consider the sum of the different species abundance influenced by their *sensitivity* to the described disturbance and by their *indicator* value (the latter being opposite to the unspecificity for any situation):

$$I = \frac{\sum_{j=1}^{n} a_j s_j v_j}{\sum_{j=1}^{n} a_j s_j}$$

being a the relative abundance, s the sensitivity value, v the indicator value and n the number of species observed in the diatom community. Thus, these indices combine the abundances of all the taxa present in a site and their individual ecological preferences to obtain a single score of water quality for a particular site. Diatom indices are calculated through OMNIDIA software [16], and scaled in a range from 1, the worst quality, to 20, the best; and they are divided in 5 water quality classes, from bad to high water quality (Table 1).

As a general trend, the highest values of the three diatom indices were in the headwaters of the different catchments and particularly in those of the Pyrenees (Fig. 3a). The lowest values were found in lowland sites, especially in those receiving high inputs of organic matter and industrial discharges. Diatom index values improved in spring, when higher water discharge conferred a higher water quality to most of the sites (Fig. 3b). The catchment with the worst water quality was the Llobregat, affected by important industrial and urban activities. The Ebre catchment had the best water quality, especially in the headwaters of Segre, Noguera Pallaresa and Noguera Ribagorçana, where some of the sites reached the value of 20 for the IBD. The three diatom indices reliably assessed the water quality of the sites and also reflected the differences between the two periods. The indices significantly correlated with the environmental variables, including the ones related with pollution and physical impact (e.g. ammonium, nitrate, phosphate, total organic carbon (TOC), general morphology, general hydrology) and those related with physiography (e.g. altitude, water velocity, water temperature) (Table 2).

Table 1 Water quality class classification of diatom indices

Index value (1–20)	Water quality
Index ≥ 17	High
$17 >$ index ≥ 13	Good
$13 >$ index ≥ 9	Moderate
$9 >$ index ≥ 5	Poor
Index < 5	Bad

It is important to stress that IPS, IBD and CEE were originally developed to assess overall water quality, not only eutrophication effects. Then, they do not completely correlate with nutrients, as other factors affecting water quality like organic matter, pH, ionic composition or salinity are influencing the indices. However, higher correlations were observed with the IPS than with the CEE or IBD. Comparing the percentages of sites in each water quality class for the three indices, it was observed that IBD tended to attenuate the extreme values (Fig. 4). Although IPS and CEE had a similar pattern, only a few sites in the high-quality class exceed the value of 18 in the case of the CEE. Thus, CEE and IBD were observed to underestimate or overestimate particular situations in the studied Mediterranean streams. Moreover, the IPS covered the whole range of values, from the lowest (1.1) to the highest (20) value. The IPS was therefore selected as the most appropriate index to assess the water quality at the studied sites.

2.3 Reference Conditions and Indicator Taxa

The reference condition approach implemented by the WFD represents a new paradigm in the biological evaluation. A basic aspect of the WFD is to base the ecological assessment of a given site on type-specific classification. A fundamental part of this approach is the determination of baseline data (i.e. reference conditions) by which to compare various disturbances and land uses for each typological group. Ecological quality ratios (EQRs) derive from this concept. EQRs are calculated as ratios of observed to expected value of the assessment method for each typology. An EQR of 1 represents reference conditions, whilst an EQR close to 0 represents a biological community that largely deviates from the community considered of reference. In each water body type, the reference conditions are represented by different sites that include the variability of the biological communities in these sites, thus including the variability in the expression of the good status. Overall, the reference condition sites represent the whole range of optimal natural conditions occurring within that water body type [17].

Following WFD requirements, the Catalan rivers were classified into *river types* and *subtypes*, and up to ten categories were defined [18]. Reference sites for each of them were established based on biological, hydromorphological and physicochemical data [19]. The classification of reference conditions should then respond to both the environmental and the biological variability. The

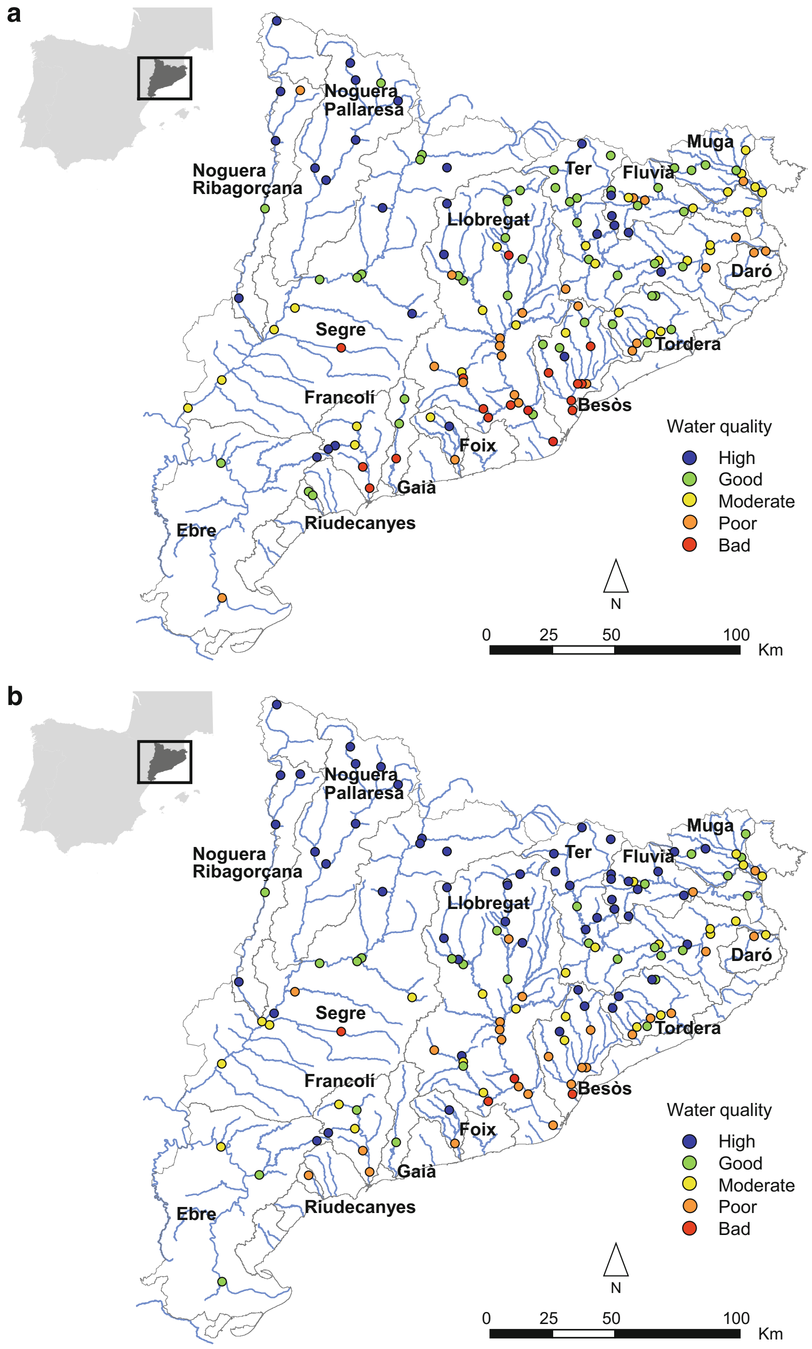


Fig. 3 Water quality classification of the sampling sites using IPS in (**a**) summer 2002 and (**b**) spring 2003

Table 2 Spearman's correlation coefficients (Spearman's rho) between diatom indices and environmental variables

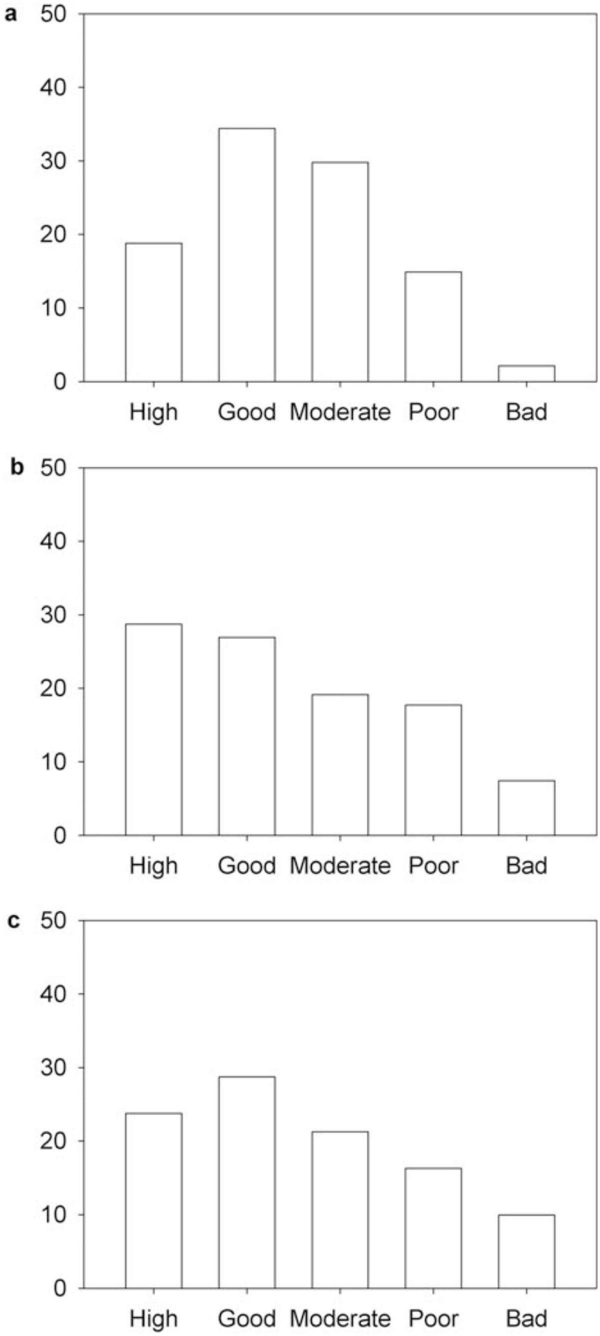
	n	IBD	IPS	CEE
General morphology	234	−0.351*	−0.379*	−0.418*
General hydrology	234	−0.253*	−0.245*	−0.276*
Riparian vegetation	234	−0.302*	−0.325*	−0.336*
Land use	234	−0.302*	−0.325*	−0.336*
Urbanisation	234	−0.229*	−0.268*	−0.294*
Agriculture	234	−0.215*	−0.210*	−0.211*
pH	264	0.226*	0.210*	0.201*
Conductivity (μS/cm)	265	−0.692*	−0.685*	−0.615*
Water temperature (°C)	261	−0.488*	−0.517*	−0.470*
Dissolved oxygen (mg/L)	162	0.406*	0.377*	0.377*
Oxygen saturation (%)	166	0.393*	0.394*	0.404*
NO_3^--N (mg/L)	216	−0.427*	−0.481*	−0.439*
NH_4^+-N (mg/L)	165	−0.439*	−0.540*	−0.552*
PO_4^{3-}-P (μg/L)	216	−0.572*	−0.607*	−0.617*
SO_4^{2-} (mg/L)	213	−0.590*	−0.602*	−0.533*
Cl^- (mg/L)	208	−0.748*	−0.762*	−0.743*
HCO_3^-(mg/L)	199	−0.474*	−0.540*	−0.450*
K^+ (mg/L)	197	−0.754*	−0.761*	−0.742*
Ca^{2+} (mg/L)	200	−0.501*	−0.535*	−0.440*
Mg^{2+} (mg/L)	200	−0.495*	−0.502*	−0.382*
Na^+ (mg/L)	200	−0.737*	−0.766*	−0.751*
TOC (mg C/L)	210	−0.562*	−0.630*	−0.646*
Width (m)	246	0.017	−0.012	−0.045
Depth (cm)	214	0.003	−0.016	−0.049
Current velocity	268	0.334*	0.285*	0.232*
Canopy cover	257	0.005	0.049	0.068
Water transparency	264	0.432*	0.399*	0.412*
Altitude (m a.s.l.)	281	0.636*	0.659*	0.651*

n number of cases
*Significant correlations at $p < 0.01$

correspondence between these a priori classifications and a classification based only on diatom data (a posteriori) was tested using 31 reference sites [20]. Diatom data was obtained for 31 reference sites using the information provided by the implementation of the WFD in Catalonia (Sect. 2.1). These 31 reference sites included three types and six subtypes of the Munné and Prat [18] classification.

The biological classification of reference sites based on diatoms comprised four cluster groups, and indicator species could be identified in all of them (IndVal [21]). There was a group that contained calcareous high- and mid-mountain streams and it was characterised by *Encyonopsis microcephala*, *Denticula tenuis* and *Cymbella excisa*. A second group of sites consisted of calcareous mid-altitude mountain streams and lowland rivers, and the most important species in this group were

Fig. 4 Percentage of sites in each water quality class for (**a**) IBD, (**b**) IPS and (**c**) CEE



Cocconeis pediculus, *Amphora pediculus*, *Navicula gregaria* and *Nitzschia inconspicua*. The third group was composed of Pyrenean siliceous and calcareous streams, and the indicator species were *Achnanthidium pyrenaicum* and *Diatoma ehrenbergii*. Sites of the fourth group were small siliceous high-mountain streams. Many of the best indicators for this group were common taxa at low water temperatures and poorly mineralised waters (e.g. *Diatoma mesodon*, *Fragilaria arcus*, *Achanthidium subatomus*, *Planothidium lanceolatum* and *Reimeria sinuata*).

The correspondence between the a posteriori biological and the a priori typological classifications was good, since the classification based on diatom communities was highly coincident with the catchment geology and altitude, like the typological classifications did. However, the classification strength of types and subtypes was weaker than the classification strength of the diatom classification [20], probably because the typological classification is mainly discriminated by large-scale geomorphological and hydrological characteristics of streams, and subtypes reflect more local in-stream features. Therefore, a combination of regional classification partially based on more local environmental characteristics was proposed by Tornés et al. [20] in order to improve the classification strength of reference conditions in Catalonia.

3 Some Lessons from the Mediterranean Intercalibration Exercise

3.1 The Intercalibration Process and Derivation of the Intercalibration Common Metric

Different intercalibration (IC) exercises between similar geographical European areas were performed in order to ensure that ecological status concepts and bioassessment methods of water quality were comparable amongst different fluvial ecosystems in Europe. The application of the EQRs against the reference conditions expresses the class boundaries between high and good ecological status and between good and moderate ecological status. Moreover, as the WFD dictates that ecological assessment has to be based on type-specific classification, member states (MS) were divided into groups sharing ecological water body types, the Geographical Intercalibration Groups (GIG). Thus, Cyprus, France, Italy, Portugal, Slovenia and Spain were the countries participating in the Phytobenthos Mediterranean Intercalibration process for rivers, using diatoms as proxies for phytobenthos.

The IC process can be influenced by sample collection and processing, taxonomic inconsistencies, choice of metrics for ecological status evaluation and criteria for reference sites selection, which can be different between the MS. In the case of diatoms, sampling collection and processing are consistently performed across Europe, following the European standards [6–9], and this removed potentially important source of ecological noise that could affect the IC process [22]. Still, as found by Kahlert et al. [23], the identification of diatoms at species or subspecies level together with constant nomenclatural changes made necessary to perform taxa harmonisation in order to assure high similarity between identifications, which consisted of a screening of inconsistencies and merging synonyms. Another aspect influencing the outcome of the IC is the different criteria for reference sites selection in the respective countries. Feio et al. [24] developed a three-step procedure for screening reference conditions in order to obtain a common dataset for reference sites for all BQE in the Mediterranean GIG. This become necessary after the results in the Central/Baltic GIG, where possible inadequate screening of data was the cause of a nutrient-related gradient within the reference samples [25]. However, rigour in the selection of reference sites did not guarantee the existence of pristine conditions in several river types, and consequently nitrate was related with diatom data in the least disturbed sites [11]. Even though four IC river types were defined for the Mediterranean [26] based on catchment area, geology and hydrological regime (Table 3), the application of the least disturbed conditions procedure showed no major differences between IC types 1, 2 and 3. Thus, data from these three types were treated together as a single type and maintained separate from type 4 (temporary rivers).

The biological classification did not correspond to the abiotic classification in the Mediterranean GIG [24]. The inconsistency between river types and biotic classification was also observed in the Central/Baltic GIG [27] and in other studies outside the context of the IC process [28, 29]. It is worth stressing that MS merged more than one national type in each abiotic IC type and in some cases one national type was split in more than one IC type, defining the wide nature of the IC types. As found in the classification of reference conditions for Catalonia [20], Feio et al. [24] suggested that other relevant variables for phytobenthos which reflect local conditions such as current velocity, substrata type, alkalinity, water hardness or light availability should be considered in order to improve the strength of the abiotic classification.

Countries participating in the Phytobenthos Mediterranean GIG used different assessment methods [11], although all of them addressed nutrient and organic contamination as main stressors. In order to compare the status class boundaries defined in each country, EQRs of the national metrics were placed on a common scale, the Intercalibration Common Metric (ICM [25]). The ICM results from the combination of the IPS [12] and the Rott's Trophic Index (TI [30]):

$$\mathrm{ICM} = \frac{\mathrm{EQR}_{\mathrm{IPS}} + \mathrm{EQR}_{\mathrm{TI}}}{2}$$

being $\mathrm{EQR}_{\mathrm{IPS}}$ = observed value/reference value and $\mathrm{EQR}_{\mathrm{TI}} = (4 - \text{observed value})/(4 - \text{reference value})$, where 4 is the maximum value for TI. Reference values for IPS and TI were calculated as the median value of reference sites for a national dataset.

Table 3 Description of the common intercalibration river types in the Mediterranean GIG

River type	River description	Catchment area (km^2)	Geology	Flow regime
Type 1	Small	<100	Siliceous	Highly seasonal
Type 2	Medium	100–1,000	Siliceous	Highly seasonal
Type 3	Small and medium	<1,000	Non-siliceous	Highly seasonal
Type 4	Small and medium	<1,000		Temporary

IPS assesses general water quality, and low IPS values corresponded to low EQR values. The TI accounts for the nutrient load impact, and it needed to be adjusted so high values represented high EQR values. Once transformed into the ICM, the national boundaries should not deviate more than a quarter of class equivalents (calculated using the high maximum EQR value for each country) from the global mean boundary value (calculated from all countries) [31]. All national boundaries in Phytobenthos Mediterranean GIG fell within the ICM boundary range, except the G/M boundary for four types (one type from Portugal and three types from Spain), which did not comply this and needed to be adjusted. Overall, it was concluded that national metrics intercalibrated were comparable [11].

3.2 Application and Constraints of the ICM to Catalan Rivers

The ICM reliably reflected the water quality for Catalan rivers (Fig. 5). Most of the sites lie between the 95% confidence bands, with the exception of a few cases. As a general trend, the Muga catchment showed the highest difference between the two indices (Fig. 5a), which corresponded also to the river typologies *Mediterranean rivers of variable flow* and *calcareous Mediterranean mountain rivers* (Fig. 5b). Differences between ICM and the national method (IPS) could reflect different value calculation for IPS in reference conditions. Reference value in the ICM was calculated as the median IPS value of all those reference sites considered in the IC and including all IC river types. Reference values for IPS were calculated at the national level (not only taking into account those reference sites used in the IC), and considering river typologies separately, this could cause the national methodology to be more precise. Then, contrasted methodologies for reference value calculation could be more striking in particular situations in the Catalan river system, as *Mediterranean rivers of variable flow* and *calcareous Mediterranean mountain rivers*, which could not be properly reflected when river typologies were not considered separately.

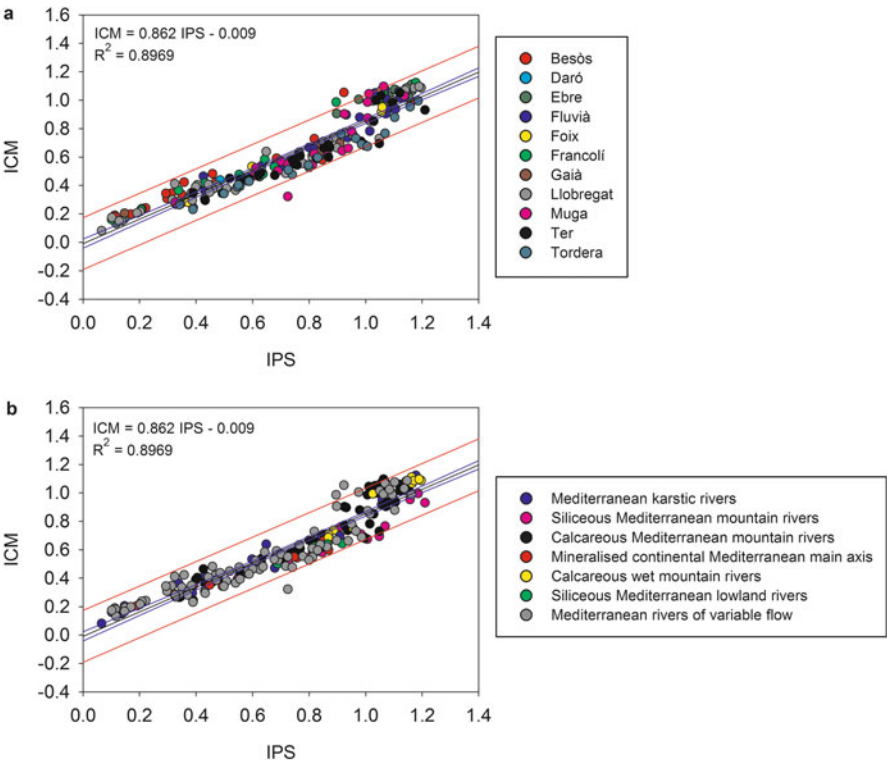


Fig. 5 Linear regression between IPS (expressed in EQR values) and the Intercalibration Common Metric (ICM), represented by (**a**) catchments and (**b**) national river typologies. *Blue lines* represent the 95% prediction bands, and *red lines* represent the 95% confidence bands

4 Conclusions

Since the European Water Framework Directive was published in 2000 [10], different diatom indices have been applied to the Catalan rivers, for their wide application (IPS and IBD) and for their interest to approach a standard for most European situations (CEE). Amongst these, the IPS was selected as the most appropriate index to assess the water quality at the studied sites. In general, the water quality for Catalan rivers is high in the headwaters of the different catchments, particularly in the Pyrenees, whilst it reaches the worst values in lowland sites of the Llobregat catchment, receiving high inputs of organic matter and industrial discharges.

The reference sites in Catalonia are mostly located in the headwaters, since it is difficult to be defined in the middle and lower river stretches. This is a general problem elsewhere in the Mediterranean region, as large rivers (catchment area >1,000 km^2) showed a small number of reference sites [11] and could not be included in the IC process. This should not be a surprising situation, since

Mediterranean rivers have a long history of human disturbances and are highly endangered ecosystems [24].

The ecological assessment is based on type-specific classification, and the classification of reference conditions should respond to both the environmental and the biological variability. In the context of the Catalan rivers, there was a correspondence between both classifications, although abiotic classifications were weaker than classification based on diatom data [20]. However, in the context of the Mediterranean IC, a disagreement between abiotic river types and types defined by biological data was found for all BQE due to the broad nature of the river types [24]. There is a debate on the degree to which local processes and geographical patterns are affecting the diatom community structure. These abiotic classifications are mainly based on large-scale geomorphological and hydrological features, and other relevant variables for phytobenthos which reflect more local conditions, such as current velocity, substrata type, alkalinity, water hardness or light availability, should be considered in order to improve the strength of the abiotic classification.

A final perspective indicates that ICM consistently related with the local-used indices (IPS), but it was also clear the need of revising the river typology as well as of revisiting the fine-tuning of taxonomic identifications. Putting effort in these aspects would improve water quality assessment at the national level and would also improve the subsequent comparability amongst countries.

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Biological Indices Based on Macrophytes: An Overview of Methods Used in Catalonia and the USA to Determine the Status of Rivers and Wetlands

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Abstract Aquatic macrophytes are commonly used as the basis for assessing the ecological condition of wetlands and rivers and are considered the basis for some of the best indicators of these ecosystems within their landscape. We review key approaches that utilize plant traits as the basis for water resource assessment, including the floristic quality assessment index (FQAI), the Qualitat del Bosc de Ribera (riparian forest quality index or QBR), indicator species analysis (IndVal), and multimetric indexes of ecological integrity (MMIs). The FQAI quantifies how "conservative" a plant species is by evaluating the degree to which it is adapted to a specific set of environmental conditions and then uses that information to assess plant community response by examining the aggregate degree of "conservatism" for all species in a community. The index codifies expert opinion a priori on the ecological nature and tolerance of macrophyte species and has been shown to be sensitive to human activities. Plant traits can also form the basis for assessment using indicator species analysis (IndVal), which allows the environmental preferences of target species to be identified and related to habitat type, site characteristics, environmental change, or gradients of human disturbance. We applied this technique to identify indicator species for river ecosystems in Catalonia. Finally, assessment approaches based on multiple plant-based metrics are illustrated. Species traits used in multimetric indexes (MMIs) are based on testable hypotheses

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about how plant communities change along human disturbance gradients. These approaches and their application to Catalan and US wetlands and rivers are explored.

Keywords Catalonia, Macrophytes, Rivers, USA, Wetlands, WFD comparison

Contents

1 Introduction

There is a strong ecological basis for using macrophytes for the assessment of aquatic ecosystems such as rivers and wetlands. Macrophytes are universal components of these systems and are key drivers of many ecosystem processes such as primary production, biogeochemical cycling, and sediment trapping [1]. Because individual species are differentially sensitive to environmental stressors, the composition of plant communities reflects the degree of stress experienced by a site and, thus, its ecological condition. Biological assessment methods are based on field data collected to allow assessment of the biotic integrity of a site by evaluating the extent to which it supports natural levels of diversity, stability (both resilience and resistance to perturbation), and the functional organization characteristic of an unstressed system of its type [2]. In contrast, ecological condition describes the extent to which a site departs from full ecological integrity; the condition is expected to decrease as anthropogenic disturbance increases [3].

Change in species diversity that results from anthropogenic disturbance is a community-level response that integrates the effects of a wide variety of environmental stressors including hydrologic alterations, excessive siltation, and nutrient enrichment. The advantages of using macrophytes as indicators for biotic assessment are many, including:

1. They are relatively large, obvious components of river corridors and wetlands.
2. They have a well-studied taxonomy with regionally specific taxonomic information for most areas.
3. Species diversity is high, allowing for the development of numerous metrics that can serve as the basis of method development.

4. Vegetation sampling methods are well developed, "low tech," and cost effective [4].

Macrophytes are also sensitive indicators due to their links to other trophic levels that ultimately affect the delivery of ecosystem goods and services [5]. For example, plants influence water quality through the uptake and accumulation of nutrients and metals in their tissues. They also act as nutrient pumps, moving compounds from the sediment to the water column. Likewise they influence the hydrologic and sediment regime through processes such as sediment and shoreline stabilization, modification of currents, and desynchronization of flood peaks [6]. Thus, shifts in plant communities correspond to shifts in the functions of a site.

The focus of this chapter is on the use of macrophytes in the assessment of biotic integrity in aquatic ecosystems, both in the USA (with emphasis on the north-central USA) and Catalonia (NE Spain). Both regions have water quality programs with well-developed biological assessment approaches and programs, including those based on macrophytes. In the USA, methods have been developed in order to implement the Clean Water Act (CWA), while in Catalonia, they were developed as part of the implementation of the Water Framework Directive (WFD). A comparison of approaches will provide useful information on the commonalities and differences in macrophyte-based assessments and illustrate their potential application in both regions. Here, we compare key approaches used to characterize plant traits as the basis for water quality assessment, including the floristic quality assessment index (FQAI), indicator species analysis (IndVal), the Qualitat del Bosc de Ribera (QBR; [7]), and multimetric indexes (MMIs, also known as indexes of biotic integrity, or IBIs).

2 Defining the Reference Condition

A key component of biological assessment is the need for an appropriate standard against which to measure ecological condition. This requires that the sites to be assessed are classified (to reduce variability within classes) and that a gradient of anthropogenic disturbance is identified. Rivers and wetlands include a wide diversity of habitats resulting in differences in the functions or ecosystem services they provide. Creating classes of similar sites within or across regions reduces variability due to the natural differences in hydrology, water chemistry, or soils. This reduces variability, making it easier to detect both the effects of human disturbance and the response of indicators.

A critical step in the development of metrics that make up assessment methods is to establish the expectations for reference condition. This is based on the reference approach presented by Brinson [8], which requires that sites be identified along a gradient of anthropogenic disturbance. Reference standard refers to the condition at the least, or minimally, impacted sites and provides the basis for quantifying the best available physical, chemical, and biological properties [9, 10] (Fig. 1). The reference condition provides the conceptual framework for relating ecological

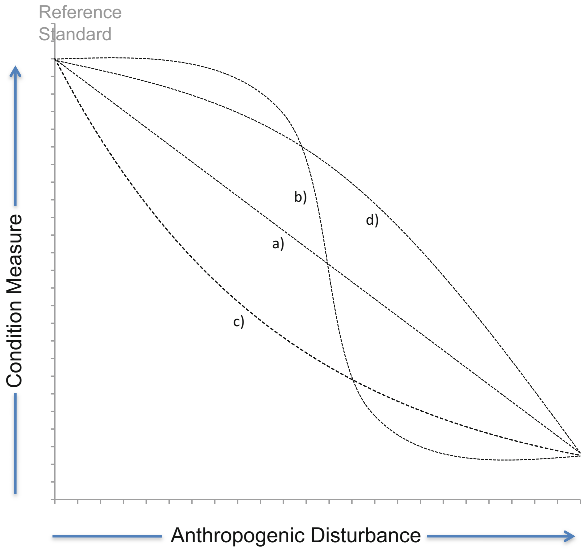

Fig. 1 Relationship between reference wetlands, a gradient of anthropogenic disturbance, and measures of condition. Reference standard refers to conditions at the least, or minimally, impacted sites: (**a**) linear response of condition to disturbance, (**b**) nonlinear response of condition to disturbance, (**c**) and (**d**) potential envelope of reference wetland condition [10]

condition and human disturbance by identifying both the high and low ends of the condition/disturbance gradient, defining the relationship between disturbance and condition, and identifying management benchmarks, for example, the condition classes that must be delineated under the WFD [11, 12]. Important distinctions include defining sites that are minimally disturbed (i.e., the ecological condition in the absence of significant anthropogenic disturbance, a difficult bar to reach in many parts of the USA or the EU), least disturbed (defined as the highest condition supported given the constraints of the landscape), and best attainable (the condition of least disturbed sites where best management practices have been implemented; [13]).

3 The Floristic Quality Assessment Index (FQAI)

The floristic quality assessment index (FQAI) is a macrophyte-based assessment method that has become a well-established means to evaluate ecological integrity in wetlands, riparian zones, and floodplains in the USA [1, 14–16]. It was originally developed by Wilhelm and Ladd [14] for the Chicago region in order to evaluate the conservation value of different sites through an assessment of the "conservatism" of the plant community. The index assesses the ecological condition or "intactness" of an area by examining the aggregate degree of ecological conservatism (or tolerance) of all species present at a site, irrespective of community type (i.e., herbaceous, forested, marsh, fen, reed swamp). FQAI scores are based on *coefficients of conservatism* (C-values), which are numerical ranks assigned to each species that indicate species' tolerance to varying environmental conditions. The

Table 1 Descriptions of the coefficients of conservatism (C-values) used to calculate the FQAI [15]

Coefficient of conservatism (C) values	Description
0	Nonnative or opportunistic native taxa that have become invasive
1–3	Taxa that are widespread and not indicative of a particular community type/high tolerance to environmental stress
4–6	Taxa that are common of an advanced successional phase/less tolerant to environmental stress
7–8	Taxa that reflect a stable community/relatively intolerant to environmental stress or human disturbance
9–10	Taxa that can successfully exist only under a narrow range of ecological conditions (intolerant to environmental stress and human disturbance)

interpretation of "conservatism" has evolved since the index was developed. Some interpret "conservatism" as the affinity of a species for habitats that represent natural, remnant areas (i.e., those with high conservation value), a view that is consistent with Wilhelm and Ladd's [14] original description [17]. However, a more common view is that conservatism represents the degree of affinity a species has for a set of specific ecological characteristics; higher degrees of conservatism result in the assignment of higher C-values [18].

C-values are based on the fact that the response of a given species to disturbance is a function of its autecological tolerance to a range of environmental conditions. Species with a narrow range of tolerance or specialized requirements have high C-values (>7) and tend to be eliminated from sites as disturbance increases. Species that can tolerate a wide range of habitat conditions or disturbance are assigned low C-values (<3). Use of the index requires that a local flora be available with coefficients of conservatism assigned to each species. In total, C-values range from 0 to 10 (Table 1) and are determined a priori based on both the ecological nature and relative tolerance of each species [16, 17]. FQAI scores are calculated based on the species present at a site irrespective of the proportional representation (evenness) of any species or its dominance, growth form, showiness, or other factors. The index is calculated using a complete species inventory as follows:

$$\mathrm{FQAI} = \frac{\sum \mathrm{CC}}{\sqrt{N}} \tag{1}$$

where

$\sum \mathrm{CC}$ = the sum of the C-value for all species identified in the area surveyed and
N = the number of native species.

Using the square root of N dampens the effects of diversity extremes, allowing naturally lower diversity, specialized, and often small areas of high ecological

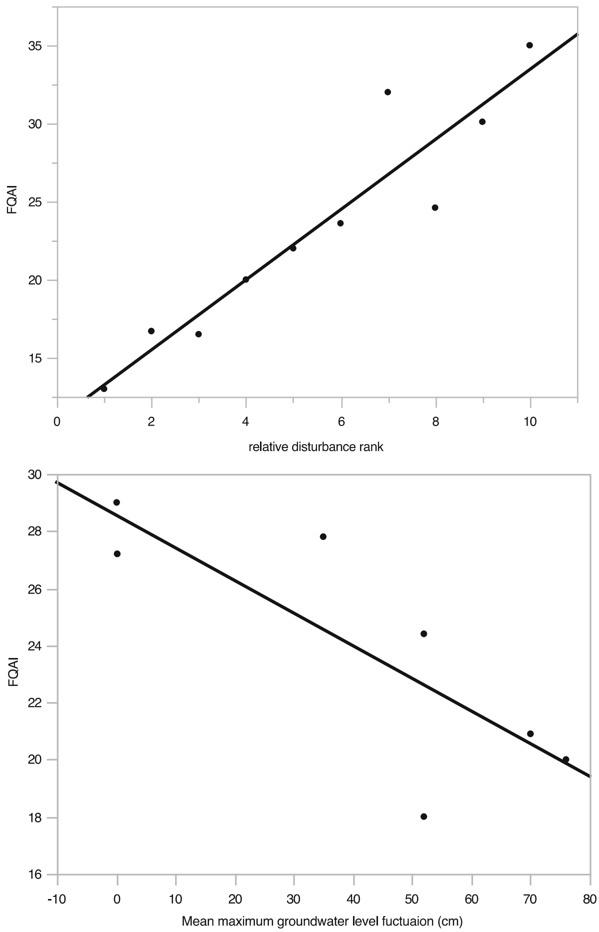

Fig. 2 Relationship between FQAI scores and (**a**) relative disturbance at a series of riparian wetlands where low scores equate with most disturbance ($y = 11.3 + 2.2{*}x$; $p = 0.001$) and (**b**) water level fluctuations at those sites ($y = 28.56–0.11{*}y$, $p = 0.025$) [20]

quality to score favorably in relation to larger sites that are often more diverse but may be of lower mean quality. The index has been shown to be effective in comparing sites regardless of plant community type and is sensitive to anthropogenic disturbance [16, 19]. For example, in an early study of riparian forests testing the responsiveness of the FQAI, sites were selected along a gradient of anthropogenic impacts and assigned a disturbance score based on:

- The land use surrounding the site
- Its land use history (e.g., had it been farmed)
- The degree of observed hydrological modification to the riparian zone and stream channel [20]

A strong correlation was found between relative disturbance and FQAI scores ($r^2 = 0.92$; $p < 0.01$; Fig. 2a). In this case, the key stressor at the sites was hydrologic modification due to a high proportion of agricultural and urban land use in the

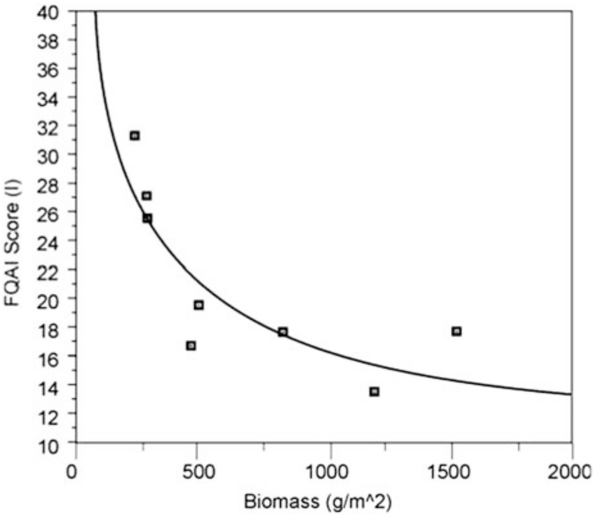

Fig. 3 Relationship between biomass production and FQAI scores in eight herbaceous wetlands in Ohio [20]

watershed, leading to increased runoff and flashy hydroperiods. The FQAI was shown to be sensitive to this with a clear link between FQAI scores and the extent of water level fluctuations ($r^2 = 0.64$; $p = 0.03$; Fig. 2b). In fact, the FQAI has been shown repeatedly to be responsive to changes in the land use surrounding a site, as well as soil nutrient levels (e.g., total organic carbon, nitrogen, and phosphorous) [16].

The FQAI has also been shown to relate to ecosystem processes, increasing its value as an indicator. For example, Keddy et al. [21] suggested that rates of primary productivity could serve as an indicator of ecological integrity, particularly in response to stressors such as nutrient enrichment. In this case, eutrophication may cause a site to be dominated by disturbance-tolerant species with monoclonal growth patterns and high productivity such as *Typha* or *Phragmites* species, resulting in low FQAI scores. As predicted, Fennessy et al. [20] found a negative correlation between FQAI scores and biomass production (itself a simple measure that integrates many processes within the ecosystem) in a study of Ohio wetlands (Fig. 3), supporting that increased primary productivity can be a sign of stress.

In a study of how changing land use affects indicators of ecological condition, Ward [22] investigated the relationships between the FQAI, other macrophyte-based indicators, and land use within a 1-km distance of each site (Table 2). Land use was quantified as the proportion of area in different land use categories (e.g., forested, agricultural, urban) as well as by an integrated land use metric, the landscape development index, or LDI [23]. The LDI was correlated with above-ground biomass production, FQAI scores, native species richness, and the percent of disturbance-tolerant species at a site (defined as those with C-values of 3 or less). The extent of urban/suburban area showed strong links with most indicators, including FQAI scores ($r = -0.64$, $p = 0.07$), percent disturbance-tolerant species

Table 2 Correlation coefficients (r) for possible indicators and land use variables for areas within 1 km of each site

Indicator	Row crop (%)	Forest (%)	Urban/suburban (%)	LDI
Biomass production	0.75**	−0.81**	ns	0.84***
FQAI	ns	0.82***	−0.64*	−0.79**
Native species (%)	ns	ns	−0.58*	ns
Native species richness	ns	0.81***	ns	−0.74**
Tolerant species (C-values 0–3) (%)	ns	ns	0.69**	0.64*
Relative cover of *Typha* spp.	ns	ns	0.68**	ns

Asterisks indicate level of significance: *$p < 0.10$, **$p < 0.05$, ***$p < 0.01$; *ns* not significant. $N = 9$ for all tests, except those of biomass production where $N = 8$ [22]

(C-values <3; $r = 0.69$, $p = 0.04$), the relative cover of *Typha* species ($r = 0.68$, $p = 0.04$), and the percent native species ($r = -0.58$, $p = 0.10$). This suggests that by integrating information on the number of species at a site and their autecology, the FQAI and associated metrics provide a measure of the stress that a site is experiencing due to landscape change [22].

An alternate use of the C-values is to calculate the mean C-value for a site and use this value either as a stand-alone index or as a metric in a multimetric index:

$$\overline{\mathrm{C}} = \left(\sum \mathrm{cc}_{ij}\right)/N_j \tag{2}$$

An advantage of the mean C is that it controls for variations in species richness more fully than do FQAI scores, and so it may be less influenced by differences in sampling area or effort. It has been shown to be correlated with anthropogenic disturbance, including functional attributes such as sediment and carbon accretion rates in headwater streams [24]. In this study, soil accretion rates ranged from 0.02 to 0.5 cm/year with the highest rates observed in floodplain depressions with a high proportion of developed land surrounding the site and lower mean C-values.

Because the FQAI has been demonstrated repeatedly to be a robust index in the assessment of ecological condition, several states in the USA now use it as part of their wetland water quality monitoring programs to make decisions about issuing permits that allow wetland impacts and to set performance standards for wetlands that must be restored or created to mitigate for those impacts [25]. More recently, the US Environmental Protection Agency (USEPA) adopted it and its associated metrics, such as the proportion of tolerant species, as core metrics in the US National Wetland Condition Assessment (NWCA) [26]. The NWCA is the first study designed to determine the ecological condition of wetlands at the national scale. The first round of sampling, which occurred in 2011, involved intensive surveys of over 1,300 sites across the lower 48 states and was carried out using a probabilistic sampling approach, which allows estimates of the ecological condition of different wetland classes with known statistical confidence.

One challenge in using the FQAI is that C-values vary regionally as a function of local conditions and the geographic range of each species. Adopting the FQAI for use at such a large scale required compilation of all C-values that have been produced for the floras of the different states and regions in the USA [26]. It also required that the coverage of C-values be expanded into regions for which no lists have been developed by considering ecoregional similarities and species distributions. The FQAI and a sensitive species metric are key metrics in a nationally applicable MMI. The survey is slated to be repeated every 5 years in order to monitor any spatial and temporal changes and to assess the efficacy of management and restoration efforts.

4 The Riparian Quality Index (QBR)

The riparian quality index, or the "Qualitat del Bosc de Ribera" (QBR), was developed in Catalonia (NE Spain) to serve as a relatively rapid assessment method for use in determining the ecological condition of riparian habitats along rivers and streams [7]. Riparian zones are critical to river functioning; therefore, their condition directly affects in-stream diversity and function [27]. Likewise, the WFD requires the use of hydrological and riparian quality elements in order to set a comprehensive ecological status for surface water bodies [11]. The QBR focuses on this aspect of river and stream ecosystems, which are often ignored in river assessment approaches. It encompasses the inherent high spatial heterogeneity in riparian communities to identify sites that are of high ecological status. As opposed to many methods that are based on in-stream biological surveys, the QBR is based on characteristics of the riparian habitat (defined as a maximum width of 50–100 m, depending on stream order). It is compiled based on scores related to (1) total vegetation cover, (2) the degree of structural (vertical) complexity of the riparian zone, (3) geomorphology (with an emphasis on features that increase plant diversity), and (4) an evaluation of river channel alterations. The overall score is used to place sites into one of five quality classes, and tests of repeatability for the QBR indicate it is robust and repeatable, in part due to its relatively straightforward structure and calculations [7].

Like many rapid assessment approaches, the QBR provides a quick, relatively inexpensive, semiquantitative measures of overall riparian zone health that complements the more quantitative and intensive methods (such as FQAI or MMIs) for assessing particular aspects of condition or stress. It has benefits such as requiring less time in the field and less taxonomic expertise than the more quantitative methods, leading to cost savings and potential for monitoring a much larger sample of sites. For these reasons, rapid methods like the QBR have a key role in the implementation of wetland monitoring and assessment programs and the effective management of the resource [3, 28].

The robust ecological rationale for the index has made it easily transferable for use in other geographic areas. For instance, while it has been tested extensively in

Catalonia where it was developed, it has also been used in southern Spain [29], in subtropical Andean streams [30, 31], in the Mediterranean regions of Australia and South Africa, and in the state of Ohio [32]. In the latter study, the QBR was adapted for use in Ohio riparian forests in order to prioritize conservation of high-quality stream reaches. Only minor adjustments were made to the index for use in this region, primarily due to the expectations for higher species richness in Ohio forests (i.e., to reflect differences in native tree and shrub diversity as well as to address the issue of widespread invasive shrubs such as *Lonicera maackii* in the eastern USA). In this study, the QBR indicated that many sites were of high quality, but for impacted sites, a common cause of degradation was a lack of connectivity with the adjacent woodlands. Fragmentation was limiting the habitat potential of these sites. This provided information for strategic management decisions to improve the habitat. In the eastern USA where riparian forests are one of the most diverse habitat types on the landscape, both in terms of species and ecosystem functions, the QBR filled a critical gap in the available assessment approaches.

5 Indicator Species Analysis

In order to implement the provisions of the European Water Framework Directive that require development of biological indicators for aquatic systems that are responsive to human-caused stressors, a diverse set of biological indices have been developed and applied in Catalan rivers [7, 33]. Because the sensitivities of different taxonomic assemblages vary, assessment methods have been developed (as described in Munné et al. [33]) based on benthic macroinvertebrates [34], diatoms (e.g., [35]), macrophytes (e.g., [36]), and fish communities (e.g., [37]). To test an additional approach using data on macrophyte communities in riverine systems, we used indicator species analysis (IndVal) to identify species that are associated with previously identified gradients of human disturbance. Disturbance was quantified using measures of water quality as well as the results of the biotic indexes used in water monitoring programs. Our goal was, in part, to examine the possibility that a small number of indicator species could characterize the ecological condition of a site as an alternative to the more holistic and intensive biological surveys [38].

IndVal analysis is a means to determine species preferences for specific environmental conditions or habitat characteristics and their potential response to changes in those conditions. Species are identified based on the breadth of their ecological niche by determining their fidelity and specificity to a series of predefined sites that are selected a priori based on their environmental characteristics [39]. These are known as vectors and can include measures of water or sediment quality (e.g., nutrients, metals, toxins), biological assessment scores, or measures of the physical habitat (e.g., temperature, particle size distribution). Data on the relative abundance (as a measure of specificity) and relative frequency (as a measure of fidelity) of species are used to determine an indicator value that describes the strength of species' association with sites that share similar characteristics [40].

Species that are associated with alterations in the structure and function of ecosystems, which show sensitivity to particular environmental characteristics, or represent a particular guild, are sound choices as indicators [38]. The IndVal approach has been applied successfully to projects with many different goals, including efforts to identify and conserve intact (low disturbance) sites, identify species that are early indicators of restoration success [41], characterize the ecological condition of a site, and monitor changes in condition and biodiversity over time [38, 42].

We tested the IndVal method to identify plant species associated with the low and high range of anthropogenic disturbance gradients in Catalan rivers as measured by vectors representing those gradients. Vectors were selected based on the availability of data and the strength of relationship of the vector to anthropogenic disturbance (all data supplied by the Catalan Water Agency). Water chemistry measures used included ammonium, phosphate, conductivity, and total organic carbon. Several rapid and multimetric index scores utilized in Catalonia for river and stream monitoring were used to indicate the level of disturbance a site had experienced. Specifically, the following biotic index scores were used as measures of anthropogenic disturbance:

- IBMWP: the Iberian Biological Monitoring Working Program [43], which measures ecological condition based on the composition of macroinvertebrate communities
- IHF: the Index de Habitat Fluvial (river habitat index) [44], based on the physical habitat of rivers and streams
- IPS: the index of specific pollution sensitivity [45], based on the composition of diatom communities to assess ecological quality

The 25th and 75th percentile vector breaks were used to designate what are considered low and high levels of human impacts (see Table 3 for a description of all vectors). Then indicator species that are associated with the high and low range of each vector were identified. Table 4 shows the indicator taxa that were identified for multiple vectors, i.e., they were common across the vector groups. Vectors based on the indexes of ecological condition, IBMWP and IPS, had the greatest number of species in common for both the low and high groups.

Species associated with minimal amounts of human disturbance (i.e., low vector range) include sensitive bryophyte species such as *Cinclidotus fontinaloides*, *Cratoneuron filicinum*, and *Pellia endiviifolia*. These species have relatively specialized habitat requirements, for example, *C. fontinaloides* prefers rocky or woody substrates in light-rich environments with limited periods of flooding. *P. endiviifolia* grows preferentially where water quality is high, often forming large patches in or near the water. In the Mediterranean region, where identifying macrophyte reference communities can be a challenge due to the relatively low diversity of aquatic species that are naturally present, the inclusion of bryophytes has been advocated to more fully represent the reference conditions [46]. The fact

Table 3 Values of the vectors that define the 25th and 75th percentiles used in the indicator species analysis (data supplied by Agència Catalana de l'Aigua)

Vector	Range of values for 0–25th percentile (low range)	Range of values for 75–100th percentile (high range)	Number of species in Group 1 (low range)	Number of species in Group 3 (high range)
Ammonium (mg/L)	0–0.1	0.2–6.7	3	8
Phosphate (mg/L)	0–0.10	0.35–2.58	6	6
Conductivity (μS/cm)	0–375	1,130–7,640	7	2
TOC (mg/L)	0–1.8	3.6–16.4	6	0
IBMWP index	0–89	179–223	5	6
IHF index	0–64	77–90	3	1
IPS index	0–13	17–20	7	3

that this indicator species analysis identified bryophytes as indicators of reference conditions supports this approach. In fact, bryophytes are used as the basis for metrics in several European macrophyte-based assessment methods used to implement the WFD [46].

In contrast, the indicator species associated with highly disturbed habitats tolerate a wide variety of conditions. Most have widespread distributions extending throughout Europe and North America. For example, *Arundo donax* (giant cane) thrives in highly impacted sites, where, for example, soils can be contaminated with heavy metals or are enriched with nutrients [47]. It tolerates high levels of human disturbance and has been included as an indicator of disturbance in an MMI developed to evaluate Iberian rivers [48]. Many of the indicator species of highly disturbed sites are floating leaved species with widespread distributions that spread rapidly, forming dense stands in eutrophic conditions. *Azolla filiculoides*, a floating aquatic fern, is particularly problematic due to its high growth rates and dense colony formation, rapidly spreading to completely cover water surfaces. It grows symbiotically with cyanobacteria that can fix nitrogen, giving it a competitive advantage particularly when phosphorus levels are high [49]. It has become a serious nuisance in Doñana National Park (SW Spain) after becoming established in 2001. Since then its population growth has been explosive [50]. Finally, both *Myriophyllum spicatum* and *Potamogeton pectinatus* (now *Stuckenia pectinata*) are aggressive invaders in the EU and the USA.

Table 4 Indicator species that are common to the low range and high range of the vectors used in the analysis of Catalonian rivers

Ammonium (low group)	Phosphate (low group)	Conductivity (low group)	TOC (low group)	IBMWP index (high group)	IHF index (high group)	IPS index (high group)
	Cinclidotus fontinaloides		*Cinclidotus fontinaloides*	*Cinclidotus fontinaloides*	None	*Cinclidotus fontinaloides*
			Cratoneuron filicinum			*Cratoneuron filicinum*
Eupatorium cannabinum	*Eupatorium cannabinum*		*Eupatorium cannabinum*	*Eupatorium cannabinum*		
		Pellia endiviifolia		*Pellia endiviifolia*		
	Rhynchostegium riparioides	*Rhynchostegium riparioides*				
			Rivularia sp.	*Rivularia* sp.		
Ammonium (high group)	**Phosphate (high group)**	**Conductivity (high group)**	**TOC (high group)**	**IBMWP index (low group)**	**IHF index (low group)**	**IPS index (low group)**
Arundo donax			None	*Arundo donax*		
	Azolla filiculoides					*Azolla filiculoides*
Cyperus eragrostis	*Cyperus eragrostis*					*Cyperus eragrostis*
	Lemna gibba			*Lemna gibba*	*Lemna gibba*	*Lemna gibba*
	Myriophyllum spicatum			*Myriophyllum spicatum*		*Myriophyllum spicatum*
Paspalum distichum	*Paspalum distichum*				*Paspalum distichum*	*Paspalum distichum*
		Potamogeton pectinatus		*Potamogeton pectinatus*		

Indicator species are shown that occur in association with two or more vectors for the low (relatively undisturbed) and high (relatively disturbed) species groups (data supplied by the Agència Catalana de l'Aigua)

6 Multimetric Indexes

Macrophyte-based multimetric indexes (MMIs) have become common tools for use in the assessment of a range of aquatic ecosystems with specific MMIs developed for fresh- and saltwater marshes, coastal marshes associated with inland lakes, forested wetlands, and riparian zones [48, 51]. They are made up of a series of metrics describing different components or functional traits of the vegetation that together reflect overall wetland condition. MMIs have been widely used for (1) establishing baseline ecological condition, (2) assessing trends in condition over time, (3) diagnosing the stressors that lead to a decline in ecological status, and (4) providing early warning signs of a change in status. The selection of metrics that make up an MMI involves testing the responsiveness of potential metrics to human disturbance [26]. A great number of metrics have been developed, corresponding to the large number of MMIs in use. Metrics can be organized into a variety of major metric types, reflecting diversity, sensitivity to disturbance, structural characteristics, and other plant traits. A key question becomes which characteristics or attributes of the vegetation should be selected as metrics in an MMI for any specific application.

In a review of the structure of the most well-established MMIs, metrics were grouped into one of ten categories in order evaluate which have the most widespread applicability (judged by how frequently they appeared in the MMIs reviewed). Categories were similar to those described above, including abundance of invasive species (nonnative), sensitive species, annual/perennial/biennial, total taxa, tolerant species, floristic quality index metrics, native graminoid, hydrophyte, aquatic guild, and invasive graminoid metrics [51]. Table 5 lists the types of metrics according to how often they have been used, reflecting their robustness and sensitivity in a wide variety of locations and habitats. These metrics are among the most universal, supporting the underlying principle of macrophyte-based assessment that, while riparian and wetland habitats may differ in terms of the species that they support, the response of these plant-based metrics to anthropogenic disturbance is similar [4, 51, 52].

7 Selecting an Assessment Approach

The choice of an assessment approach depends on how the data will be applied. Fully reaching the goals of the WFD or the CWA depends on the evaluation of the ecological status of aquatic sites. Here, we have discussed four approaches, and we conclude by providing a brief overview of the pros and cons of each for the purposes of ecological assessment.

FQAI – The use of the FQAI and its associated metrics (mean C) is complicated by the need for a regional flora with the coefficients of conservatism (C-values) for all species. This is not a small investment, requiring time and the expertise of

Table 5 Categories of plant metrics ranked according to how often they were used in a survey of 20 different assessment methods (the number of times each metric type was used is indicted)

Rank	Metric category (number of times metric used/20 methods evaluated)	Comments
1	Invasive or nonnative species metrics (20/20)	• Used in all MMIs evaluated
2 and 3	Sensitive species metrics (18/20)	
	Annual/perennial/biennial metrics (18/20)	
4	Total taxa metrics (17/20)	• Include metrics related to total richness by plant zone
5 and 6	Tolerant species metrics (16/20)	• Include nutrient- and turbidity-tolerant metrics
	Floristic quality assessment index (FQAI) metrics (16/20)	• Include FQAI score, cover weighted FQAI, and mean C
7	Native graminoid metrics (13/20)	
8	Hydrophyte metrics (12/20)	• Include “wetness metric” (%similarity of wet value weighted for abundance)
9 and 10	Aquatic guild metrics (11/20)	• Aquatic guilds used in MMIs designed for lakes and deeper water communities
	Invasive graminoid metrics (11/20)	

Note that a higher rank does not necessarily indicate a more responsive metric [51]

botanists to compile and agree on the assignments. Once C-values have been determined however, the FQAI is relatively easy to use (provided the user has the appropriate botanical expertise), and it can be completed relatively quickly. Most importantly, it has been repeatedly shown to be highly sensitive to anthropogenic disturbance, which makes it an excellent candidate for assessment programs (although its ability to diagnose specific stressors is limited). It has been adopted by several states in the USA as a means to implement water quality standards under the CWA, either on its own or as part of an MMI, and several government agencies in the USA now use it for monitoring ecological condition, as does the federal USEPA. Unfortunately, C-values have not yet been developed for Catalonia or other areas of the Mediterranean basin; this is an investment that will have to be made in order to use this powerful index.

QBR – As a rapid assessment approach, the QBR has many advantages such as requiring less time in the field and less taxonomic expertise than more quantitative methods, which can lead to cost savings and potentially larger sample sizes. It is based on the assumption that the condition of stream corridors increases as their physical and biological structural complexity increases. Thus, the QBR is robust, as witnessed by the ease with which it has been transferred to other regions for use in assessment programs. As it is currently constructed, however, its use is limited to riparian zones along streams and rivers and is not designed to assess wetlands, although modifications to the method might make this possible.

Indicator species analysis (IndVal) – Identifying indicator species is a powerful approach in assessing the response of plant species to specific stressor gradients. However, the analysis requires a large amount of data up front, both on the species composition of a relatively large number of sites and on the quantitative measures of potential stressors at each site (soil chemistry, water quality, etc.). The resources needed to perform these surveys can be prohibitive. However, once identified, indicator species are valuable for their ability to diagnose stressors that are the cause of decreased ecological status. In addition, IndVal results, along with threshold analysis, can be used to determine the minimum level at which human activities alter the ecosystems. Overall, the indicator species approach has not been fully tested in monitoring programs nor has it been adopted for use in the implementation of the WFD or CWA.

Multimetric indexes – MMIs are the most widely adopted approach in the ecological assessment of streams and rivers, wetlands, and lakes. Plant-based MMIs are perhaps less common than those developed for other biological assemblages (e.g., invertebrates, fish, diatoms), but there are a wealth of plant MMIs in use and a large number of metrics that have been developed and tested. These provide the foundation for the development of MMIs for new regions. The strength of this approach is that a range of plant traits can be assessed by different metrics, providing an integrated response of the community to human activities. An associated weakness is that while some combinations of metrics perform better than others, the underlying ecological explanation for this is not well understood. Ultimately this is a common and successful approach that has been widely adopted in the USA, with great promise for use in Catalonia. In the USA, scoring thresholds are typically developed for good, fair, and poor ecological status (to meet the requirements of the CWA); five ecological quality classes could easily be defined as per the WFD.

In sum, ecologically sound assessment methods are a critical component of ecological protection programs. The choice of assessment method depends on the region in which it will be used, the resources available, and the application of the data. The well-developed science behind macrophyte-based assessment will aid in reaching the goals of restoring and maintaining fully functional aquatic sites on our landscapes.

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Fish as Ecological Indicators in Mediterranean Streams: The Catalan Experience

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Abstract The Water Framework Directive includes fish fauna as one of the biological elements, jointly with aquatic flora and benthic invertebrates, to assess and monitor water and habitat quality. Successful implementation of the Directive depends in part on the development of reliable, science-based tools to directly assess biological conditions. Although fish have been used as ecological indicators for more than 30 years around the world, mainly in North America and more recently in Europe, few studies have been done in Mediterranean streams. Fish assemblages of the Mediterranean basin, similarly to other Mediterranean areas such as California, have particular characteristics that hamper IBI's development: few native species, poor knowledge of their ecological requirements, high number

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of endemisms with a wide range of tolerance to environmental variations and many exotic species. This chapter summarizes our experience in developing fish-based tools in Catalonia. We discuss the challenges and difficulties to develop these approaches in Mediterranean streams. We show the IBICAT2010 as a fish-based assessment method suitable for the evaluation of the ecological status of Catalan rivers. Moreover, we assess size-related variables as a bioassessment tool because population size structure can provide insights into species-specific applications and management. Finally, we analyse the longitudinal connectivity throughout Catalan rivers and fish passes by using the index of river connectivity (ICF) specially designed to Catalan rivers.

Keywords Biological indices, Ecological status, Freshwater fish, Human pressure, Monitoring program, River connectivity, Water Framework Directive

Contents

1 Fish as Ecological Indicators

Fish have some particular features and advantages as indicators of the health of freshwater ecosystems [1]. Fish continually inhabit the receiving water and integrate the chemical, physical and biological histories of the waters. Most fish species have long lifespans (about 2–10 years) and can both reflect long-term and current water quality. The sampling frequency needed for trend assessment is less than for short-lived organisms, and the taxonomy of fishes is well established, enabling professional biologists the ability to reduce laboratory time by identifying most specimens in the field. Fish have large ranges and are less affected by natural microhabitat differences than smaller organisms, making them extremely useful for assessing regional and macrohabitat differences. Moreover, fish are highly visible and valuable components of the aquatic community to the public, making communication easier.

It is widely known that exposure to environmental stressors (e.g. pollution or low oxygen) causes detrimental effects on important fish features such as metabolism, growth, resistance to diseases, reproductive potential and, ultimately, the health, condition and survival of fish [2–4]. Depending on the intensity and duration of stress exposure and species-specific features these negative effects may be transferred from the individual to population or community levels [5]. The knowledge, for each species, of their functional attributes, range of tolerance and responses in front of different kinds of stress will permit to use freshwater fish as ecological indicators. The biological indicators complement the traditional physicochemical indicators, facilitating a better assessment and management of freshwater ecosystems.

Although the study of fish as ecological indicators started at the beginning of the twentieth century [1], it is not until the year 1981 that James Karr proposed [6] the first biological index based on fish, namely the Index of Biotic Integrity (IBI). James Karr defined biological integrity as “the ability to support and maintain a balanced, integrated, and adaptive community of organisms having a species composition, diversity and functional organization comparable to those of natural habitats within a region” [7]. Different factors (both biotic and abiotic) may affect this biotic integrity: chemical variables, flow regime, biotic factors, energy source and habitat structure (Fig. 1). The original IBI consists of 12 fish-assemblage attributes (called metrics) that reflect predominant anthropogenic effects on streams. Each metric describes a particular taxonomic, trophic, reproductive or tolerance feature of the assemblage. An IBI score represents comparisons between metric values at a sampling site and those expected under conditions least affected by anthropogenic disturbance.

The utilization of the fish as bioindicators has spread all over the planet, and the original version of the IBI has been modified in numerous ways for application in many different regions and habitat around the world [8–11]. These new versions maintain a multimetric structure but they incorporate different typologies, number of metrics and values. A European project (FAME: Fish-based Assessment Method for the Ecological Status of European Rivers) developed the European Fish Index (EFI), the first standardized fish-based assessment method applicable across a wide range of European streams [12, 13]. Because of several limitations observed in the performance of the index, a new version (EFI+) was developed [14]. Although many IBIs have been adapted for different European countries and specific rivers basins [15, 16], few of them are used on Mediterranean streams because they present a number of potential difficulties.

2 Challenges and Difficulties in Mediterranean Streams

The fish assemblages of the Mediterranean basin, similarly to other Mediterranean areas such as California, have particular characteristics that hamper IBI’s development: few native species, poor knowledge of their ecological requirements, high number of endemisms with a wide range of tolerance to environmental variations and many exotic species [17, 18]. The Index of Biotic Integrity mainly has been developed in areas with complex fish communities: many native species with different trophic levels. The IBIs characterize by having many metrics (normally around 12), independent among them (metrics with redundant information should

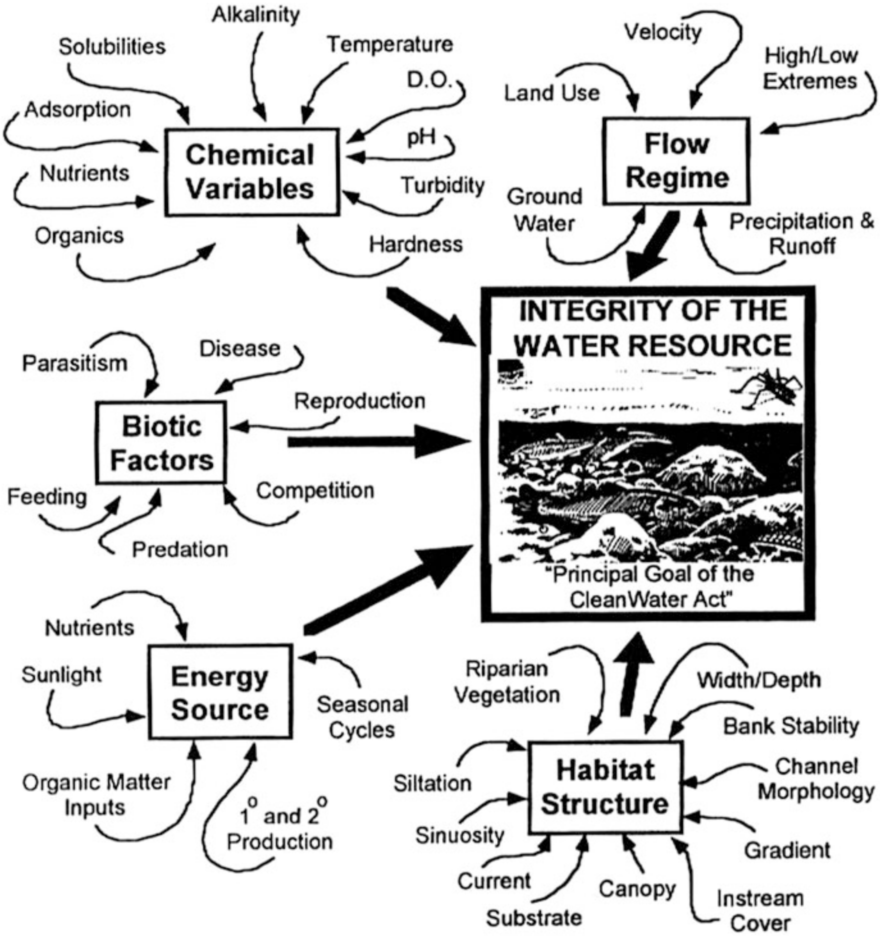

Fig. 1 The five principal factors, with some of their important chemical, physical and biological components that influence and determine the integrity of water resources. From [1]

be avoided) and of different levels of organization (individual, population, community, ecosystem and landscape) [8, 19]. In order to correctly detect different kinds of ecosystem alterations, Karr and Chu [19] emphasized that IBIs should have metrics for each organization level.

Developing enough metrics for IBIs is difficult in Mediterranean rivers due to low fish richness [20]. Miller et al. [8] suggested that although an IBI with less than 12 metrics may work, it may be less responsive to a broad spectrum of degradation. The low fish richness in the Mediterranean basin hinders the use of very common fish metrics such as: diversity of species, trophic specialization and reproductive strategies. This low richness is especially problematic in headwater sites, where often there are only one or two species and sometimes one is an introduced or translocated salmonid [17]. In a project in the Tordera River, in

Catalonia, we studied this situation and recommend that this low richness could be compensated assessing metrics based on age or size structure, fish individual state or including other aquatic biota [21]. For instance, there are IBIs that combine fish metrics with benthic invertebrates [22] and both adults and tadpoles of amphibians [17]. With regard to individual health, although DELT anomalies (deformities, eroded fins, lesions and tumors) are incorporated in many IBIs [23], the presence of ectoparasites or fish condition is not included. Both metrics have been shown as a good bioindicator in different studies in Catalonia watersheds in cases with high contamination with heavy metals [24], anoxic situation [25] and alteration of natural flow regime [26]. These fish metrics could help to increase the number of metrics in Mediterranean IBIs, concretely at individual health level [19], because they have responded significantly in front of habitat degradation and poor quality of water.

Hydrological variability of Mediterranean-type regions profoundly determines the life forms and life cycles of aquatic organisms, as well as ecological processes [27, 28]. Fish fauna from these heterogeneous ecosystems must frequently survive under alternating scenarios of too much or too little water with a few intermediate but crucial periods of investment in recruitment and growth [20]. Under these conditions, fishes tend to have short life spans, rapid growth rates, high fecundity and early sexual maturity and spawning, as well as generalist and opportunistic feeding strategies [29, 30]. The native species of Mediterranean streams have a wide range of tolerance to environmental variations and are habitat and feeding generalists, well adapted to survive in changing environments [20, 31]. Many metrics of IBIs describe a particular trophic, reproductive or tolerance guilds of fish species. In Mediterranean streams, sometimes it is difficult to classify the species due to its wide range of tolerance and in many cases relatively little is known about the ecology of many fishes in Mediterranean areas [32, 33]. More basic studies of the ecological requirements of Mediterranean species are needed to fill this information gap. Long-term studies monitoring both fish assemblages and physicochemical parameters could be invaluable in this regard.

The introduced species are a serious environmental problem in Mediterranean freshwater ecosystems and a challenge to develop an IBI [34, 35]. Some authors suggest that exotic species should not be included in the absolute richness metrics of IBIs [36, 37] but could be a reliable indicator of poor river health [38]. Moreover, other authors indicate that although exotic species are a loss of biotic integrity they might provide a great deal of information about water and habitat quality [17]. This represents a conflict between using an IBI to measure diversity and abundance of native organisms and using an IBI to measure water and habitat quality. Exotic species have been incorporated in metrics of different IBIs applied in Mediterranean basins [20, 39, 40]. Although Ferreira et al. [41] included exotic species in the metric of absolute richness, in other works where this metric was present only native species were considered [20, 39]. While Sostoa et al. [39] and Magalhães et al. [40] suggest some metrics exclusively for native species (e.g. number of native insectivores), in general exotic and native species are pooled in metrics of trophic and reproductive functions. Although to take the information about water and habitat quality that exotic species provide this could be a solution in front of the problem of low native species in Mediterranean basin. In our opinion valuing positively the abundance and richness of exotics fish is counterproductive. Nevertheless, in Catalonia that could constitute a problem since the number of native fish species currently sampled (Table 1) is similar to the exotic ones (Table 2). A total of 16 native species were sampled (2007–2008) in front of 16 exotic species.

Table 1 List of native fish species in the Catalan rivers and their IUCN Red List categories

Species	UICN categories
Petromyzon marinus[a]	VU
Acipenser sturio[a]	CR
Anguilla anguilla	VU
Alosa alosa[a]	VU
Alosa fallax[a]	VU
Atherina boyeri[a]	VU
Platichthys flesus[a]	LC
Syngnathus abaster[a]	LC
Salmo trutta	VU
Cottus hispaniolensis[a]	CR
Gasterosteus aculeatus	EN
Barbatula quignardi	VU
Cobitis calderoni[a]	VU
Cobitis paludica[a]	VU
Achondrostoma arcasii	VU
Barbus haasi	VU
Barbus meridionalis	VU
Gobio lozanoi	LC
Luciobarbus graellsii	NT
Parachondrostoma miegii	NT
Phoxinus bigerri	VU
Squalius laietanus	VU
Tinca tinca[a]	LC
Aphanius iberus[a]	EN
Valencia hispànica[a]	CR
Salaria fluviatilis	VU
Pomatoschistus microps	LC
Chelon labrosus[a]	LC
Liza ramada	LC
Mugil cephalus	LC

[a]Absent in the last sampling period carried out in Catalan rivers, 2007–2008. Taxonomy and status of species are assigned following [32]

Table 2 List of introduced fish species in the Catalan rivers and their origin

Species	Origin
Acipenser baerii[a]	Europe
Oncorhynchus mykiss	North America
Salvelinus fontinalis[a]	Europe
Abramis brama[a]	Europe
Abramis bjoerkna[a]	Europe
Alburnus alburnus	Europe
Carassius auratus	Asia
Cyprinus carpio	Asia
Pseudorasbora parva	Asia
Rutilus rutilus	Europe
Scardinius erythrophthalmus	Europe
Misgurnus angillicaudatus	Asia
Esox lucius	Europe
Lepomis gibbosus	North America
Micropterus salmoides	North America
Perca fluviatilis	Europe
Sander lucioperca	Europe
Ameiurus melas	North America
Ictalurus punctatus[a]	North America
Silurus glanis	Europe
Gambusia holbrooki	North America

[a]Absence in the last sampling period carried out in Catalan rivers, 2007–2008). Taxonomy and status of species are assigned following [32]

Finally, another difficulty to develop metrics and IBIs in the Mediterranean basin is the lack of reference areas to test the metrics. In order to know the ecological status the current condition has to be compared to the natural conditions (structure, composition, function, diversity) in the absence of human disturbance or alteration (reference condition) [42]. Chovarec et al. [43] suggest that "reference condition is the state that has existed before the human interferences, or at least without human influences that have altered significantly their natural characteristics". Owen et al. [44] consider that the "reference condition is when physical-chemical, hydromorphologic and biological values corresponding to the area without human alteration". The concept of reference condition is widely known and used. For example the EPA (US Environmental Protection Agency) in the United States [45], the "National River Health Program" in Australia [46], the "River Health Programme" in South Africa [47] and the "Water Frame Directive" in Europe use the concept of reference condition to assess the ecological status and to develop fish metrics and indices. The problem in many regions of the world, and especially in the Mediterranean basin, is that pristine sites are unavailable due to an intensive human activity during many centuries [48, 49]. Not only are undisturbed sites unlikely to exist but the rate of stream modification has been accelerating in recent decades [33]. While the "least disturbed" or "best available" sites are sometimes used as alternatives to reference sites [50], the WFD requires pristine or near pristine reference sites [51]. However, it is almost impossible to find Mediterranean stream reaches where native fish assemblages have not been altered. Especially in areas as river mouths it can be difficult to know the original biota composition and also the natural flow regime, because reservoirs and water abstraction have profoundly altered them. In these cases biologists have to use different methods to select the reference conditions. One of the methods is the "expert criterion", which is easy but requires an exhaustive validation [44]. In other cases, researchers may use predictable models and paleolimnology information or in some cases must rely on historical data, collected when human activity was low, to define reference condition [19, 52, 53].

One extreme case of the problem in reference conditions is the case of reservoirs. Due to their artificial nature natural reference conditions do not exist for reservoirs. For this reason different authors have adapted the original fish metrics of Karr et al. [36] and suggested to name it the RFAI (Reservoir Fish Assemblage Index) [54, 55]. In Catalonia, the fish assemblages of 14 reservoirs were sampled by boat electrofishing in the littoral and multi-mesh gillnets in the limnetic zone [56]. Most eutrophic reservoirs were dominated by common carp (*Cyprinus carpio*) whereas oligotrophic reservoirs presented other fish species intolerant to pollution rather native (such as brown trout, *Salmo trutta*). The absolute and relative abundance of common carp was strongly related to the trophic state of the reservoir and 40% of its variation was explained by total phosphorous concentration. Despite clear changes in species composition, there was no significant effect of water quality on overall fish richness or Shannon's diversity, suggesting that for such low richness assemblages species composition is a better indicator of cultural eutrophication of reservoirs than fish diversity. WFD considers reservoirs as artificial water bodies or heavily modified water body, therefore in these cases the aim is to obtain a good ecological potential before 2015. In the WFD the good ecological potential of one artificial water body is defined as the nearest values to the most similar natural water body.

3 Features of Fish in Catalonia

Mediterranean streams have flow patterns strongly seasonal: low flow in the hot summer drought and flash floods during autumn and spring storms [27]. During the summer, some parts of the stream can remain reduced as a series of pools. Interannual variability in precipitation is high while lengthy periods of drought are common [27]. This hydrological variability of Mediterranean-type regions profoundly determines the life forms and life cycles of aquatic organisms, as well as ecological processes [20]. Besides these natural factors, the water resources of the Mediterranean basin suffer a high human pressure because it is a highly populated area with urban and industrial growths, especially in the last 50 years [57–59]. Apart from the direct pressure on the water resources, Catalan rivers, and other rivers in the Mediterranean basin, also suffer from alterations in natural hydromorphology and riparian vegetation [60, 61]. Although the pollution from industrial and urban waste has in general decreased thanks to entry in operation of many treatment plants [62], there is an increase in the number of contaminants of emerging concern, particularly from pharmaceuticals, personal care products and perfluorinated compounds among others [63].

In Catalonia, with the exception of the Ebro, rivers have a basin area of intermediate dimensions (from 312 km^2 of Foix to 4,948 km^2 of Llobregat) and average streamflows that oscillate between 1.5 m^3/s of Francolí and 20 m^3/s of Llobregat. Most rivers are strongly regulated in middle and lower reaches but even in upper parts. In Catalonia there exist more than 20 big dams and nearly 8,000 of big obstacles according to the database of the Catalan Water Agency. Current information of fish populations in Catalonia comes from two periods of sampling programme (2002 and 2007–2008) to develop some fish based IBI during the implementation of the WFD [39, 64]. For the historical data we are based on many published and unpublished records of other authors and ourselves. At present, the icthiofauna of Catalonia is formed by a total of 51 species of which 30 are native (14 of them endemic for the Iberian Peninsula) and 21 are non-native or exotic (Tables 1 and 2). Many of native species are migratory: four anadromous, one catadromous and seven amphidromous. Fourteen of the native species are absent during last sampling period (2007–2008) [64] due to different situations. Sampling was made on riverine water bodies, but not in transitional or coastal waters, which means that the four amphidromous species less tolerant to freshwater were not present. The absence of *A. iberus* and *V. hispanica* may be explained by the same reason because both species live in freshwater and brackish littoral lagoons. But the absence of the other eight native species is related to the high number of threatened for freshwater fish commonly found in Mediterranean basins [18].

There is a threat of extinction for most part of the native fish species of the Catalan rivers, and two of them (*A. sturio* and *P. marinus*) are locally extinct. Other species are closer to local extinctions, for example *C. paludica*, and some others have patchy distributions, *G. aculeatus* or *S. fluviatilis*, which present fragmented populations with smaller distribution areas than historical ones. According to the evolution of their distribution, we can compare the number of basins with the presence of native species (Table 3). Just for one species (*B. haasi*) the number of basins with presence shows no changes comparing to their historical range. Many others are present in a smaller number of basins. *G. aculeatus* is of particular concern for their extremely reduced distribution and *S. laietanus* due to their long-term decline. A particular case for native species is the group of species which are increasing the presence on more basins as a consequence to translocate to others basins for sport fishing purpose (*B. barbatula*, *G. lozanoi*, *L. graellsii*, *P. miegii* and *P. bigerri*).

Table 3 Evolution of fish distribution by basins according to the historical presence, for the period 2002–2003 [39] and for the period 2007–2008 [64]

	Number of basins (historical)	Number of basins (2002–2003)	Number of basins (2007–2008)
Native species			
Anguilla anguilla	14	8	10
Barbatula barbatula	1	2	2
Barbus haasi	7	7	7
Barbus meridionalis	7	6	6
Gasterosteus aculeatus	5	3	2
Gobio lozanoi	1	4	4
Luciobarbus graellsii	1	4	5
Parachondrostoma miegii	2	4	5
Phoxinus bigerri	2	6	6
Salaria fluviatilis	4	2	3
Squalius laietanus	11	9	7
Salmo trutta	3	10	8
Exotic species			
Misgurnus anguillicaudatus	0	0	2
Alburnus alburnus	0	4	6
Ameiurus melas	0	1	2
Carassius auratus	0	3	4
Cyprinus carpio	0	8	10
Esox lucius	0	1	1
Gambusia holbrooki	0	5	5
Lepomis gibbosus	0	6	6
Micropterus salmoides	0	2	2
Oncorhynchus mykiss	0	5	3
Pseudorasbora parva	0	1	2
Rutilus rutilus	0	1	5
Sander lucioperca	0	1	1
Scardinius erythrophthalmus	0	3	4
Silurus glanis	0	1	2

Related to exotic species (Table 2), Catalan rivers are a hot spot and the main origin and introduction route to the Iberian Peninsula [34, 65]. Some of the previously detected species are not established (e.g. *A. baerii* or *I. punctatus*) but there are new additions to the exotic fishes like *M. anguillicaudatus* [66]. More than 50% of the exotic species have increased their distribution with respect to the previous period like *R. rutilus* (present in four more basins) or *A. alburnus* (present in two more basins). Some other species maintain their presence in the previously detected basins (*E. lucius*, *G. holbrooki*, *M. salmoides* or *S. lucioperca*). All exotic fishes are more related to lotic conditions present in reservoirs of Catalan rivers

[56]. Most of the species collected in Catalan reservoirs are exotics (11 species), from which *C. carpio* and *R. rutilus* are the most abundant in the lowest altitude reservoirs [56]. Fish introductions are still growing with new species on the list and in expansion for the naturalized exotics. For that reason, many river stretches are far away from the biotic integrity (just about one third of sampled localities) and many others are dry without fish live (another third of the sampled localities) [39, 64]. On the other hand, some improvements, mainly in water quality, imply an increase of native fish density. The recovery of the population of *A. fallax* in the Ebro river during last years could confirm this idea [67].

4 IBICAT 2010

The IBICAT_{2010} is a fish-based assessment method suitable for the evaluation of the ecological status of Catalan rivers [64]. It is an improved version of the IBICAT [39]. The IBICAT_{2010} is a type-specific method that is based on eight environmental variables (altitude, slope, Strahler river order, mean annual air temperature, mean July air temperature, mean annual rainfall, mean July rainfall, distance to river mouth) that were selected as the best descriptors of a river classification based on the historical fish distribution. A discriminant analysis classification was used for the classification of each site. The overall misclassification rate was 0.16. A total of six river types were defined: type 1 – coastal streams; type 2 – humid mountain; type 3 – main courses; type 4 – Mediterranean lowland; type 5 – high mountain; type 6 – main courses of large rivers. Metrics describing the composition, abundance, functional traits, age structure and health condition of the fish fauna were first screened through a Pearson correlation analysis between each metrics and a synthetic pressure index based on water quality, hydromorphological alteration and habitat quality variables. Non-significant correlations were not allowed. Then, to evaluate the response of the candidate metrics to pressures a graphical analysis (boxplots) supported by statistical tests (ANOVA) was performed. Finally, redundant metrics were removed based on Spearman correlations: metrics pairs with $\rho \geq 0.9$ were not allowed. The whole screening process was performed for each river type. A total of 17 metrics were selected: density of alien species, density of native marine migratory fish, density of native piscivorous, density of intolerant species with less than 15 cm total length, density of invertivorous, density of omnivorous, density of rheophilics, number of alien tolerant species, number of native intolerant species, number of lithophilic native species, percentage biomass of native benthic, percentage individuals with injuries/deformities/parasites, percentage of intolerant species, percentage of omnivorous species, percentage of introduced invertivorous species, percentage of native lithophilic species and percentage of native tolerant species.

The IBICAT_{2010} is computed with a specific metric subset per river type using the formula:

$$\mathrm{IBICAT}_{2010} = -\sum (Mt \times R) + K,$$

where Mt is the value of the metric, R is the correlation coefficient between the metric and the global pressure and K is the constant of the river type that allows a minimum IBICAT_{2010} value that is not negative but near zero.

Finally the IBICAT_{2010} value is categorized into five quality classes, as defined in the EU WFD. The scoring criteria used to define the classes followed the procedure proposed by the European working group REFCOND [68].

5 Size-Related Variables as a Bioassessment Tool

As we discussed in second section of this chapter, the difficulties to develop enough fish metrics in IBIs on Mediterranean streams could be compensated by assessing metrics based on age or size structure. In particular, body size is a key property of organisms and arguably the most important trait affecting the ecological performance of individuals [69]. The implications of body size on growth, mortality and trophic interactions highlight the importance of size structure for population and community functioning [70–72]. Population and community size structure is considered a good health indicator because it has the potential to inform on whether disturbance is affecting the population and, moreover, it can help to identify the complex effects of biotic and abiotic influences [36, 73]. At least two studies have been developed in Catalonia focusing on size structure as a bioassessment tool in Mediterranean streams [26, 74].

Murphy et al. [74] focused their assessments of population size structure responses to anthropogenic perturbation on chub (*Squalius laietanus*), one of the most widespread native stream fish in Mediterranean basin. They studied the anthropogenic perturbation on 311 sites across Catalonia, including local data on stream condition and landscape indicators of degradation, via principal component analysis. Anthropogenic perturbation in streams was collinear with altitudinal gradients and highlights the importance of appropriate statistical techniques. Of the population size structure metrics explored, average length was the most sensitive to anthropogenic perturbation and generally increased along the disturbance gradient. Changes in variance, kurtosis and skewness were weak. The unexpected increases of mean *S. laietanus* body size with anthropogenic perturbation, strong effects of river basin, collinearity with spatial gradients and the species-specific nature of responses preclude the direct application of size structure in freshwater bioassessments.

Also significant results on size structure were found in a study of ecological impacts of small hydropower plants on headwater stream fish [26]. They studied the effects of water diversion of 16 small hydropower plants on fish assemblages and habitat features in the upper Ter river basin, which has headwater reaches with good water quality and no large dams but many of such plants. In the control reaches they detected higher average fork length and total weight, higher fish abundance and better fish condition than in impacted reaches, although the results were species-specific. Accordingly, species composition was also affected, with lower relative abundance of brown trout (*Salmo trutta*) and Pyrenean minnow (*Phoxinus bigerri*) in the impacted reaches and higher presence of stone loach (*Barbatula quignardi*) and Mediterranean barbel (*Barbus meridionalis*). Brown trout was the only fish species that has its size-related variables changed significantly.

Although the application of size-related variables in fish-based freshwater bioassessments appears difficult, population size structure can provide insights into species-specific applications and management.

6 Assessing Longitudinal Connectivity and Fish Passes

Currently, most fish can no longer migrate to complete their life cycle in Catalonia, Europe and most of the world because their natural habitats were modified by human activity. River obstacles cause direct effects on population biology, such as local extinctions due to a lack of dispersion and recolonization, genetic isolation, non-accessibility to spawning or feeding areas, refuges from predators and shelter areas or sites for harmful environmental conditions – i.e. pollution, big floods, droughts or other human disturbances and natural disasters [75]. Migrating fish upstream of reservoirs and large rivers are also an important contribution of food for other species, such as otter [76]. Existence of rivers with poor connectivity is considered one of the major causes of declines in many continental fish species in Iberian Peninsula [32, 77], Europe [78–80] and worldwide [81].

Transverse obstacles in river beds cause serious ecological consequences because they block the natural flow of water, sediments and biota. In Mediterranean regions, water abstraction may change a perennial stream to an intermittent one, increasing the duration and magnitude of droughts and limiting the stream's ability to support aquatic biota.

Restoration of fish migration should pay proper attention to dam and weir removal, which is the most environmental positive solution at medium and long term [81]; a total restoration of river longitudinal connectivity is only possible by demolishing obstacles [82]. If the obstacle cultural value or its current use (hydro-power, irrigation, etc.) does not allow their removal, the promotion of close-to-nature fish passes, such as lateral channels and fish ramps, which provide optimum conditions for a wider range of species, individuals and flows [83], should be carried out. Rehabilitation measures should ensure the re-establishment of at least a good ecological status of rivers according to the European Water Framework Directive (2000/60/EC). This rehabilitation should include effective fish passages, but also habitat recovery and connection with well-preserved source areas [82]. Similarly, implementation of environmental flow regimes is urgently needed because without this, other measures could be useless.

Reestablishment of river connectivity became a legal requirement under the Water Framework Directive (2000/60/EC) and the European Plan for Eel Recovery (Regulation 1100/2007). It also is extremely important for the conservation of endangered freshwater species included in the Habitats Directive (92/43/CEE). However, the capacity of native fish fauna to use fish passes and their natural patterns of movement are still poorly understood [83]. Moreover, fish pass assessments could provide important knowledge regarding fish movement patterns [75, 84].

The presence of 886 big obstacles (according to the Catalan Water Agency database), mostly small weirs and some dams, seriously affects migratory fish species into the Catalan rivers. Migration routes of fish, some of which Iberian endemisms, were damaged. Large migratory fish, as European eel (*Anguilla anguilla*), are not present upstream of the dams in Catalonia. Twaite shad (*Alosa fallax*), European sturgeon (*Acipenser sturio*) –which is locally extinct – and sea lamprey (*Petromizon marinus*) populations are similarly affected [32, 39], while other non-diadromous fish have also had their migration routes negatively impacted and are consequently now endangered. Moreover, transforming rivers into a series of ponds especially benefits foreign fish [85].

During the period 2006–2010, a study of fish pass facilities in Catalonia was carried out through direct inspection of 93 detected fish ways present in 10.6% of the total obstacles [86] (Fig. 2). Especially retro-fitted solutions using broad-spectrum technical structures, mainly pool fishway or pool pass facilities, were located. Most of them were mainly in the Pyrenees to improve trout fisheries.

The existing solutions in Catalonia to improve fish migration have been in some cases insufficient, and where they do exist, fish passes can be poorly maintained, or insufficient, for all of the native fish fauna from each water body. Less than one-half (36% of total) of fish passes are currently reliable for all native fish in Catalan rivers. With some exceptions, fish passage rates were quite low; only those species with great ability to overcome obstacles – such as salmonid – or larger individuals of other fish groups were able to migrate [86]. Currently, there are few examples in Catalonia of weir removal and close-to-nature fish passes (Table 4).

This situation was quite equivalent, for example, in Australia in 1985, when it only had 44 fish passage devices for the thousand obstacles present throughout the country, most of which were poorly maintained and generalized unable to practice all native fish species [89]. The same happened in other European countries, such as France [90], the UK [91] and the Netherlands [92] until the 1990s.

To compare the stream flow and the fish assemblage in different basins, a team of Girona University [93] selected gauging stations in middle reach, downstream of reservoirs; they also estimated the "naturalized flow" (i.e. the flow expected if there was no direct human influence on the watercourse, e.g. from water abstraction) using the Sacramento Soil Moisture Accounting (SAC-SMA) model, a flexible, well-known model initially developed by Burnash et al. [96] and widely used by the US National Weather Service and also the Catalan Water Agency. The SAC-SMA model is a concept-based rainfall-runoff model, with areal precipitation and potential evapotranspiration as inputs. The assessment of hydrological alterations,

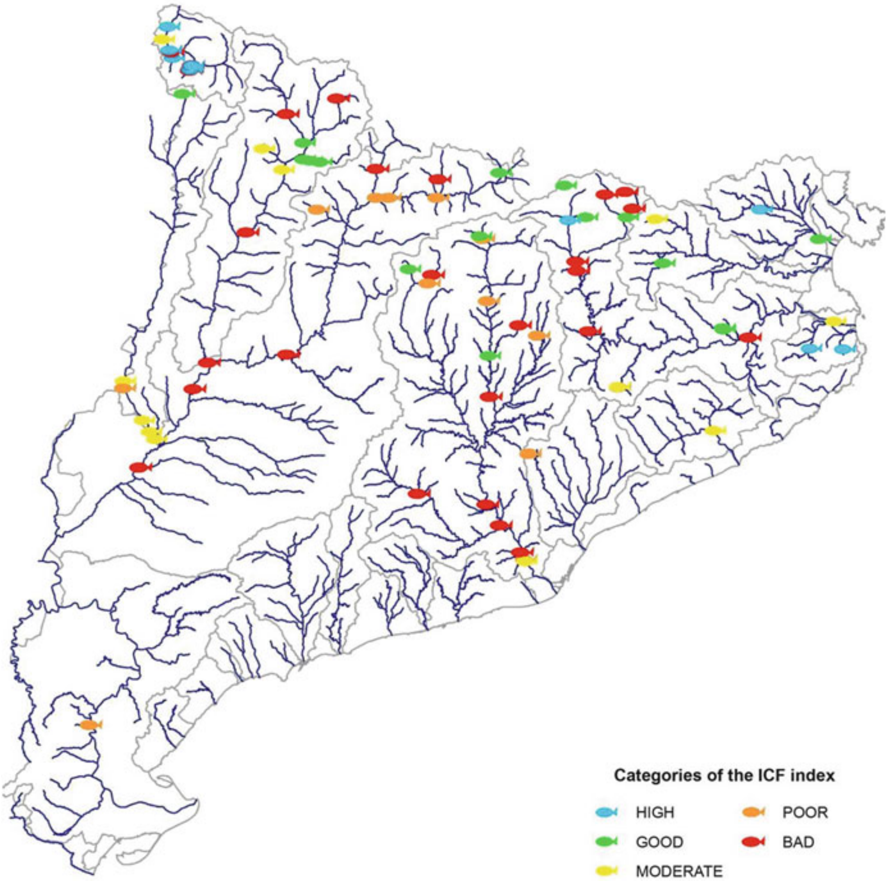

Fig. 2 Fish passes from Catalonia in 2010 [86] and results of the index of river connectivity (ICF index) [87] of each

following the Kappa index, among others, also has been done by members of the Lleida University [94].

Fish pass effectiveness was almost assessed in Catalonia following useful previous criteria for Mediterranean rivers [77, 86, 88]: (1) general data collection, using rapid assessment techniques, including the calculation of the ICF index (River Connectivity Index) [87], to evaluate the theoretical degree of impediment for fish passage; (2) indirect estimation techniques, using trapping fishing systems and/or electric fishing systems (CEN standard norm *UNE-EN 14011:2003*) to compare fish population structure between river sections [79, 84, 95]; mark-recapture methods and individual mark-recapture methods, using Passive Induction Transmitters (PIT tags) at many sites; and (3) direct estimation techniques, installing fish traps at the water intake of the fish pass to compare fish population structure and fish crossing rates with potentially migrating downstream fish

Table 4 Existing connectivity solutions and typologies of fish passes in the rivers of Catalonia in 2010

Solutions		Number
Restoration solutions	Total obstacle removal	1
	Partial obstacle removal	15
Close-to-nature solutions	Fish ramps	7
Broad-spectrum technical solutions	Pool fish passes	34
	Pool fish passes without drops	3
	Slot passes or vertical slot fishways	9
	Deflectors	8
	Denil or baffle fish passes	2
Mechanical or specific technical solutions	Eel ladders	6
	Siphons and fish pumps	2
Other solutions not considered effective	Smooth ramps	6

Adapted from [88]

population, obtained using electric fishing systems, complemented by daily collection of hydrological and environmental data, mainly using fish crossing rates and deviations of size frequencies [75, 84]. In some places, despite being limited by water turbidity and the presence of a large number of migrating fish, visual counts [83, 95] have been done as well.

The index of river connectivity (ICF, from the Catalan name *Índex de Connectivitat Fluvial*) [87], designed and improved by members of the Catalan Water Agency in collaboration with the Center for the Study of Mediterranean Rivers – Ter River Museum (CERM), evaluates the theoretical degree of impediment for fish passage and is based on comparison between physical characteristics of the obstacle, the fish pass (if any) and the swimming and/or jumping skills to overcome the obstacle of the potentially native fish fauna present in an evaluated river section. The ICF is divided into three blocks that encompass assessment of the obstacle and the fish pass as well as the estimation of certain modulators. Finally, the ICF classifies connectivity into five levels from very good to bad depending on the degree of permeability for different fish groups, discriminating among infrastructures based on the chance they can be crossed by all species, only by some species, or by no species.

The ICF was tested for 101 transverse obstacles in rivers of Catalonia, obtaining representation of the five expected quality levels (from very good to bad, Table 5), and it is considered coherent with the real permeability of the obstacles. Its ease of application compared to in situ measurements of fish movements and the detailed information recorded by the index make it a very useful tool for the diagnosis of the longitudinal connectivity of rivers and for guiding measures for hydromorphological quality improvement. In addition, due to the variety of species and hydrological regimes addressed and solutions used to date, it is essential to complement this quick assessment technique with the determination of the in situ fish pass effectiveness of any new solution implemented.

Table 5 Quality classification of connectivity for obstacles with and without fish pass solutions adapted from [87]

	With pass solution (%)	Without pass solution (%)	Total (%)
Very good	17 (21)	0 (0)	17 (17)
Good	12 (15)	6 (29)	18 (18)
Moderate	15 (19)	0 (0)	15 (15)
Poor	11 (14)	7 (33)	18 (18)
Bad	25 (31)	8 (38)	33 (32)
Total	80	21	101

Advancing the understanding of fish movement patterns will require regularly the monitoring of the efficiency of the principal fish migration solutions. For fish ways situated in key locations, for example, in the lower parts of rivers, because of their importance for amphidromous, anadromous and catadromous fish species, it would be appropriate to adapt fish pass structures to enable the installation of large permanent fish traps, as has been performed in many European countries, especially those that have important salmon or eel fisheries, or automatic fish counting devices (e.g. based on electric resistivity, infrared light and/or video camera system).

In many Catalan rivers, four fish metrics (catch per unit effort, number of benthic species, number of intolerant species and proportion of intolerant individuals) distinguished between sites impacted and unimpacted by water abstraction [93]. These four significant fish metrics, and probably others (number of insectivore species, number of native species, number of families), may be used to assess rivers suspected to have problems with abstraction. Some of these fish metrics are already used in existing European IBIs. In particular, low collective values of these fish metrics may warn of substantial hydrologic alteration [93].

Otherwise, the concordance between indexes of hydrological alteration and the IBICAT2010 index [64] which assess the ichthyofauna analysing the obtained results using the Kappa index in the rivers of Catalonia [94] is low. The indexes of hydrological alteration do not serve to assess hydrological impacts on fish community as they apply a much longer time scale and may not reflect specific changes in specific months or years. However, there is a slight relationship between these two indexes for dry years: dry years have major hydrologic alteration of the water bodies and a greater relationship with the number of present fish [94].

Regarding the existent fish passes in Catalonia until 2010 [86], all of them being broad-spectrum technical structures, their assessment indicates that brown trout (*Salmo trutta*), which exhibit a high capacity to overcome obstacles by swimming and/or jumping [90, 91], seem to be able to migrate upstream using the different types of fish passes present. However, these results show that if fish pass waterfalls are higher than 0.2 m and/or fish pass water velocity is higher than 2 m/s, only the largest individuals of species with a moderate capacity to overcome obstacles, including Mediterranean mullets (*Liza ramada*, *Mugil cephalus* and *Chelon labrosus*) and some cyprinid species, such as Ebro barbel (*Luciobarbus graellsii*; FL >55 mm), Western Mediterranean barbel (*Barbus meridionalis*; FL >0.13 m), Iberian redfin barbel (*Barbus haasi*) and Ebro chub (*Squalius laietanus*), are able to cross upstream. Moreover, if a fish pass waterfall is a maximum height of 0.1 m and/or a water velocity of less than 0.5 m/s, the results show that most species and individuals can use the fish pass, including small species with a low capacity to overcome obstacles, such as Pyrenean gudgeon (*Gobio lozanoi*), Pyrenean minnow (*Phoxinus bigerri*), European eel (*A. anguilla*) and youngs-of-the-year of other species including brown trout (*S. trutta*), Ebro barbel (*L. graellsii*) and Western Mediterranean barbel (*B. meridionalis*; FL<0.09 m). Finally, important movements of fish are mostly associated with particular spawning periods and/or periods just after high or moderate peak flows, as has been indicated in many other studies [75, 83, 90]. This finding also supports the idea that fish pass evaluation should be performed, at least, at times of maximum activity of different fish species, i.e. early spring for mullet species, spring for cyprinids and autumn for salmonids.

Close-to-nature fish passage assessment is almost pendent in Catalan rivers. However, information is already available, and positive, from two fish ramps. The fish ramp of the Teula's weir of the Ter River at Manlleu was evaluated in May 2012 and May 2014. With an ICF index [87] of 85 and the fish species size frequencies downstream and upstream being similar, it supposes a small barrier effect and a good fish pass effectiveness. The associated fish ramp at the gauging station of the Fluvià River at Olot (EA013) has been assessed between spring and autumn 2013. With a score of 95 of the ICF index, it allows the passage of all native fish species from this river. However, complementary actions at entire watershed scale are required to improve river connectivity in both cases, especially to recover European eel (*A. anguilla*) from sea to source, as happens in most Catalan river basins.

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Fish-Based Indices in Catalan Rivers: Intercalibration and Comparison of Approaches

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Abstract Freshwater ecosystems are among the most affected by anthropogenic disturbances, and fish have several advantages for monitoring them, such as the response at larger temporal and spatial scales and its visibility to the society. This chapter summarizes our experience in developing fish-based indices in Catalonia. We describe some differences observed among crews in electrofishing captures and habitat assessments. We also analyzed the suitability of a single pass for conventional monitoring in the region and differences in capturability among sites and species by comparison with multiple passes and block nets. Furthermore, we summarize the results of two contrasting approaches, a site- and a type-specific one (IBICAT2a and IBICAT 2b) applied to Catalan rivers. The site-specific was not successful and further data are needed for its improvement. A protocol for the

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computation of a type-specific, multimetric index (IBICAT2b) is given. The IBICAT2b fish index uses 4–8 metrics depending on river type and has been validated with environmental pressures both throughout Catalonia and the whole Ebro River basin. An Excel file is also given as an online supplementary material for the computation of this fish index.

Keywords Biotic integrity, Catalonia, Ecosystem health, Fish biotic index, Rivers, Spain, Water Framework Directive

Contents

1 Introduction

Freshwater ecosystems are severely threatened from human-generated pressures, including water abstraction, pollution, construction of reservoirs, and invasive species. The continuous deleterious effects of human pressures have promoted the need for biological monitoring as well as the development of biological indices [1–3]. Fish are among the taxonomic groups with more longevity in aquatic environments and are excellent ecological indicators for a number of reasons [4]. Fish assemblages have been shown in a number of regions to respond to anthropogenic disturbances including flow regulation (e.g., [5]), habitat fragmentation [6], water pollution [7], land-use change [8], hydrological alteration (e.g., [9]), and acidification [10].

One disadvantage of using fish as ecological indicators is that their population densities are more difficult to estimate accurately and their catchability depends on a number of factors including electrofishing equipment, the characteristics of the river reach [11–13], and species-specific features such as morphology or behavior [14, 15]. The estimation of catchability and intercalibration of data are important to combine data from different fishing teams and to develop protocols for future work or monitoring [12]. Habitat quality is often assessed during fish sampling [16, 17] and inconsistency of habitat assessment among researchers has been also reported by several researchers (e.g., [18–22]).

This chapter summarizes our experience in developing fish-based indices in Catalan Rivers [23, 24] and synthesizes our studies: (1) to estimate the effects of fishing crew and other factors on fish catchability and the resulting fish metrics and on habitat assessments and (2) to attempt to develop type-specific- (i.e., IBICAT2b) and site-specific-based indices (spatially-explicit approach) (i.e., IBICAT2a). We also aim to give a protocol and an Excel for an index (IBICAT2b) that has been validated throughout Catalonia and recently throughout the whole Ebro River basin (Bae et al. unpublished data).

2 Comparison of Electrofishing Crews

Understanding the differences of catchability is particularly important for intercalibration of fish data from various research groups as well as computing fish indices. Several studies have been conducted to balance the compromise between representativeness of fish assemblage in the sampling area and sampling cost (e.g., time, staff, and expenditure), including the comparison of single- vs. multiple-pass electrofishing over various habitats (e.g., [25–30]), and the analysis of electrofishing equipment type (e.g., [31]) and suitable sampling length [30, 32–37]. However, little attention has been paid to assess the differences of catchability among electrofishing crews and equipment and the effects of sampling frequentation in Mediterranean regions.

We compared capture efficiencies based on standard fish descriptors (abundance, observed fish richness and species composition) obtained from four different fishing crews in Mediterranean streams [12]. In eight sites at headwater and middle reaches of a Mediterranean river, we sampled fish in two adjacent stations which had the similar habitat condition at each site using two different methods (single-pass electrofishing without block nets vs. four-pass electrofishing with block nets). During the first fishing day, two different methods were applied, but during the rest of the days only the single pass was applied in order to compare the effects of the consecutive sampling on fish abundance and assemblage structure. We applied a Williams' crossover design, which is based on a Latin square design and is characterized by that (1) all crews are assigned only once to each sampling site during the four consecutive sampling days; (2) all crews are equally distributed; (3) it allows to test for potential carryover effects. We analyzed the differences in species richness, abundance, and proportional abundances due to the different catchability by the four research teams using generalized linear models (GLMs) with Poisson errors and log link functions (species richness and abundance) or binomial errors and logit link functions (proportional abundance). We also applied the software EstimateS (http://viceroy.eeb.uconn.edu/EstimateS) to estimate richness based on the removal estimates (i.e., four-pass electrofishing) using the second-order jackknife richness estimator (Jack 2; [38]), which is one of the most widely recommended estimators. Furthermore, we estimated population sizes and capture probability for the most abundant species in the four-pass electrofishing using program MARK using four different multinomial models (i.e., a model with constant catchability between different electrofishing passes (P), a model with constant catchability between electrofishing passes (P1), a model with nonconstant catchability between electrofishing passes (P1L), and a model with nonconstant catchability between passes and a quadratic function of fish length (P1L2)). These models were compared using Akaike's information criterion [39].

Our results indicated that single-pass electrofishing was effective in the study area. It captured a large percentage of abundance (40–60%) as well as species richness (50–100%). Unsurprisingly, electrofishing was more efficient upstream than downstream and all species were generally captured in sampling sites with few species (i.e., headwaters). Furthermore, even though it is more difficult to detect all species in mid-river sections with higher species diversity, single electrofishing showed also high catchability there. Although observed species richness was not significantly influenced by the use of block nets, average CPUE was significantly higher using block nets. In addition, observed species richness was not significantly influenced by the research team, fishing day, or carryover effects. However, total CPUE depended on fishing day, crew, carryover effects, and site. Catchability varied depending on species, size, and removal passes.

In summary, single-pass electrofishing can be adequate to estimate abundance, species composition, and richness in headwaters and middle courses of this Mediterranean region. However, various methodological factors (e.g., reach length, number of passes, fish size, and species) influence electrofishing capture efficiency. Our results also show that the effectiveness of electrofishing depends on fishing crews because of different personal skills and practice. Therefore, electrofishing sampling protocols (e.g., sampling time and effort and equipment type) should be standardized as much as possible to get comparable data [24].

3 Comparison of Habitat Assessments Among Sampling Teams

The assessment of habitat quality is essential in fish studies because each fish species often has specific habitat requirements [40] and altered habitats are considered a major disturbance in aquatic ecosystems [41]. Therefore, habitat assessment has been developed as an integral part of stream biological monitoring [42–45]. However, because habitat assessments are often based mostly on visual observations or a minimal amount of measurement [45], the variability of assessments frequently occurs among researchers (even experienced ones). We compared the differences in scoring the habitat characteristics among four research teams. Each research team conducted the habitat monitoring with the same protocol at each site after finishing the electrofishing described in the previous section. Each team surveyed hydromorphological descriptors, riparian vegetation, aquatic vegetation, refuge type, observed visual impacts, land use, and habitat based on a

Table 1 Comparison of descriptors of habitat assessment among assessors. Degrees of freedom = 2 and 9

Categories	Variables	Type III sum of squares	F	P	Partial η^2
Hydromorphology (mesohabitat)	% Riffle	756	7.61	0.01	0.63
Hydromorphology (mesohabitat)	% Glide	687	2.84	0.11	0.39
Hydromorphology (mesohabitat)	% Pool	170	1.57	0.26	0.26
Hydromorphology (substrate)	% Bedrock	37	3.65	0.07	0.45
Hydromorphology (substrate)	% Boulder	148	0.62	0.56	0.12
Hydromorphology (substrate)	% Cobble	682	6.37	0.02	0.59
Hydromorphology (substrate)	% Gravel	827	6.57	0.02	0.59
Hydromorphology (substrate)	% Sand	28.5	0.61	0.56	0.12
Hydromorphology (substrate)	% Silt and clay	1.95	0.15	0.86	0.03
Hydromorphology (hydrology)	Average width	0.08	0.43	0.66	0.09
Hydromorphology (hydrology)	Full bank height	0.84	1.43	0.29	0.24
Riparian vegetation	% Marginal riparian cover	309	0.8	0.48	0.15
Riparian vegetation	% Areal cover	78	0.16	0.85	0.03
Riparian vegetation	% Trees	1,315	4.42	0.05	0.5
Riparian vegetation	% Shrubs	1,596	6.33	0.02	0.58
Riparian vegetation	% Grass	3,600	13.5	0.00	0.75
Aquatic vegetation	% Macrophyte cover	160	0.76	0.49	0.15
Aquatic vegetation	% Helophytes	233	1.59	0.26	0.26
Aquatic vegetation	% Hydrophytes	73.6	1.46	0.28	0.24
Aquatic vegetation	% Floating leaves	30.6	1.6	0.25	0.26
Aquatic vegetation	% Floating plants	892	1.95	0.20	0.3
Aquatic vegetation	% Algae	4,988	1.72	0.23	0.28
Refuge type	% Total refuge	5,526	6.78	0.02	0.6
Refuge type	% Structural shelter	67.6	0.1	0.91	0.02
Refuge type	% Caves	523	1.11	0.37	0.2
Refuge type	% Aquatic vegetation	168	2.42	0.14	0.35
Refuge type	% Submerged riparian vegetation	474	3.52	0.07	0.44
Refuge type	% Trunk and branches	45.1	1.08	0.38	0.19
Observed impacts	Muddy water	1.22	4.95	0.04	0.52
Observed impacts	Stones with black bottom	0.06	0.64	0.55	0.13

(continued)

Table 1 (continued)

Categories	Variables	Type III sum of squares	F	P	Partial η^2
Observed impacts	Channelization	0.25	1.5	0.27	0.25
Observed impacts	Erosion	1.06	4.28	0.05	0.49
Observed impacts	Highways, roads, etc.	0.56	1.43	0.29	0.24
Land use	Forest use	0.31	1.6	0.25	0.26
Land use	Agricultural land use	1.95	8.37	0.01	0.65
Land use	Residential land use	0.56	1.43	0.29	0.24
Habitat	Microhabitat score	0.31	0.3	0.75	0.06
Habitat	Habitat diversity (macrohabitat)	0.56	0.2	0.82	0.04
Habitat	Channelization	2.78	1.47	0.28	0.25
Habitat	Channel morphology	0.31	0.07	0.93	0.02
Habitat	Flow	0.62	2.65	0.12	0.37
Habitat	Degree of clogging	6.72	8.01	0.01	0.64
Habitat	Margin erosion, R	10.6	8.36	0.01	0.65
Habitat	Margin erosion, L	12.3	5.62	0.03	0.56
Habitat	Aquatic veg. (macrophytes)	0.06	0.02	0.98	0.01
Habitat	Riparian veg. (R margin)	2.78	0.39	0.69	0.08
Habitat	Riparian veg. (R margin)	9.81	2.9	0.11	0.39
Habitat	Width of riparian veg. (R margin)	15	3.28	0.09	0.42
Habitat	Width of riparian veg. (R margin)	25.5	8.61	0.01	0.66

veg vegetation

modified version of the US Rapid Bioassessment Protocol (RBI) [46] for Mediterranean rivers (Table 1), which was used during the sampling of the project to implement the Water Framework Directive (WFD) in Catalonia [23, 24, 47]. Table 1 shows the list of habitat assessment descriptors as well as the significance of the differences among four assessors and a measure of effect size (partial η^2). Of 49 habitat assessment descriptors, 12 were significantly different among the four research teams that assessed them independently ($P < 0.05$). Percentage of grass in the riparian vegetation showed the highest difference among research groups (Table 1, Fig. 1), and four variables (i.e., degree of clogging, erosion of margins (right and left), and width of riparian vegetation (left margin)) from the Rapid Bioassessment Protocol, which provides a detailed protocol to score these features, were also different among the four assessors. A multivariate test suggested that although overall differences among assessors were not significant (MANOVA Wilks' λ, $F_{2,\ 18.5} = 5.482$, $P = 0.165$), probably due to low power, they were

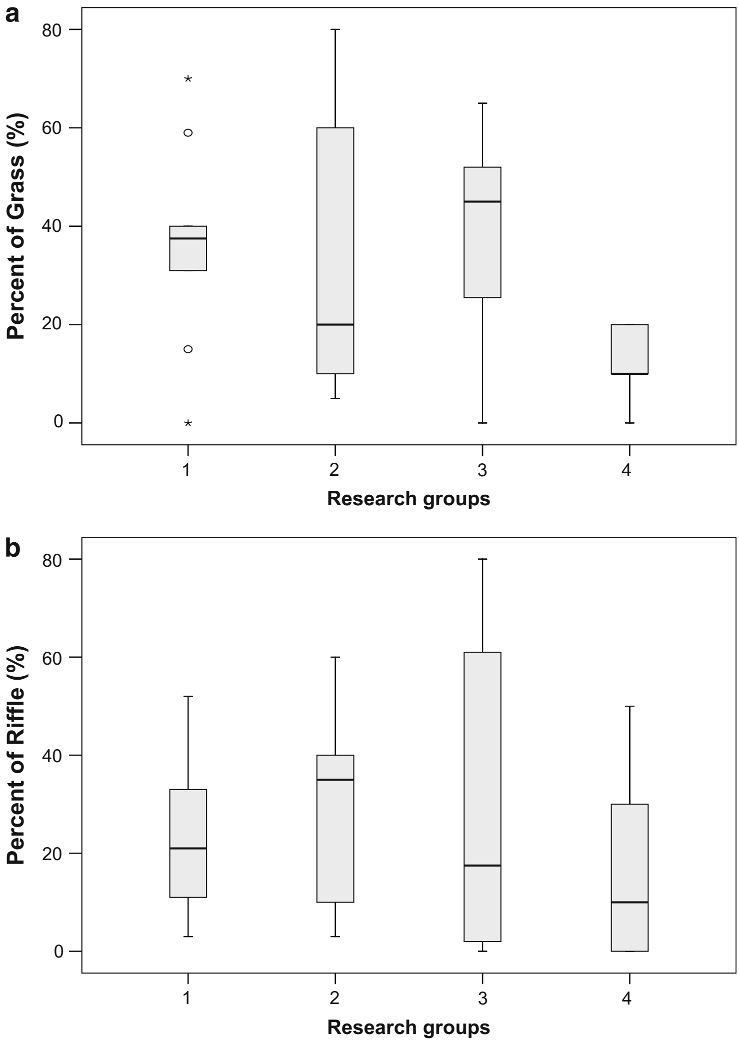

Fig. 1 Box plots of the scoring of % grass and % riffle among four research groups (see Table 1 for statistical analysis). Each box corresponds to 25th and 75th percentiles; the *dark line* inside each box represents the median; error bars show the minima and maxima except for outliers (*open circles* or *asterisks*, corresponding to values >1.5 box heights from the box)

more important (partial η^2 of 0.980 vs. 0.907) than differences among sites, which were significant ($F_{99,\ 18.5} = 3.698$, $P = 0.001$) and very clear.

Roper and Scarnecchia [19] reported that although consistency of habitat quality evaluation is improved with uniform training, inconsistency increases among researchers, as the habitat types to be classified become more diverse. Hannaford et al. [45] showed that even if the evaluation of habitat assessment becomes similar among groups after equal training in a certain type of habitats, large differences are still observed in other habitat types. Our results also suggest that the scoring for habitat assessment can be highly inconsistent among different research groups even using the same habitat assessment protocol. Therefore, habitat assessment requires more clear and detailed criteria and more training to make a similar evaluation among groups.

4 Development and Comparison of Fish Indices: Type- vs. Site-Specific Approaches

In addition to IBICAT_{2010} (see [4] in this book), whose development was led by Nuno Caiola, two other approaches (i.e., a type-specific and site-specific) were attempted in Catalan rivers [23]. Type-specific fish indices are based on a classification of sites in a region on homogenous types based on environmental or faunistic features and use different metrics and scorings in the different areas. On the other hand, site-specific approaches do not use a classification and instead predict the reference fish metrics from the environmental features of the sites [48, 49].

The WFD requests that various biotic assemblage descriptors (e.g., metrics) should be integrated into a single index to assess ecological status [3, 50]. These indices should represent the status of impairment in a research area [51–54]. Community metrics (e.g., number of intolerant species) and trophic guilds (e.g., percentage of piscivores), which group species sharing a common ecological trait into a single variable, have been commonly applied to develop bioassessment metrics based on fish assemblages [52, 55] (Table 2). It is assumed that these traits respond to anthropogenic disturbances consistently across a wide spatial extent [53, 54]. In addition, unlike species composition, which varies strongly across regions and biogeographical areas [56], patterns from functional traits are mainly determined by environmental filtering (e.g., [55, 57–61]).

Most predictive models evaluating ecological status start from comparing the biotic condition at current sampling sites with the expected biota without anthropogenic disturbance or in reference conditions [49, 62, 63]. Thus, changes in biotic condition from anthropogenic disturbance can occur only when the range of variation (or response) in reference (natural) conditions is well known [64, 65].

In this section, we summarize the two approaches (i.e., a site-specific one, IBICAT2a, and a type-specific one, IBICAT2b) based on the same guild classification for the fish fauna of Catalonia (Table 2), which was based on a comprehensive literature review. Fish development was based on a database of 364 sites in Catalonia, visited during 2007–2008, of which 8 sites could not be sampled due to the excessive discharge, 45 sites were dry, 76 sites were sampled but no fish was captured in them, and 235 sites were sampled with fish captured. At the 311 sampled sites, the total number of species (NST) ranged from 0 to 13 (median = 2, mean = 2.3), the number of native species (NSN) was from 0 to 8 (median = 1,

Table 2 Features of the freshwater fish fauna from Catalonia used for development and computation of the indices

Family	Species	Tolerance	Feeding habitat	Habitat	Reproduction	Feeding group	Migration	Longevity	Status
Acipenseridae	*Acipenser sturio*	I		RH	LITH	OMNI	LONG	LL	A
Anguillidae	*Anguilla anguilla*	T	B			PISC	LONG	LL	A
Balitoridae	*Barbatula quignardi*		B	RH	LITH	BENT		SL	A
Blenniidae	*Salaria fluviatilis*		B		LITH	INSV		SL	A
Centrarchidae	*Lepomis gibbosus*	T	WC	LI		INSV		SL	I
Centrarchidae	*Micropterus salmoides*		WC	LI		PISC		LL	I
Clupeidae	*Alosa alosa*	I		RH			LONG	LL	A
Clupeidae	*Alosa fallax*	I		RH			LONG	LL	A
Cobitidae	*Cobitis bilineata*							SL	I
Cobitidae	*Cobitis calderoni*	I		RH		INSV		SL	A
Cobitidae	*Cobitis paludica*	T		RH		INSV		SL	A
Cobitidae	*Misgurnus anguillicaudatus*	T	B	LI		OMNI		IM	I
Cottidae	*Cottus hispaniolensis*	I	B	LI	LITH	INSV		SL	A
Cyprinidae	*Alburnus alburnus*	T	WC			OMNI		SL	I
Cyprinidae	*Achondrostoma arcasii*		WC					SL	A
Cyprinidae	*Barbus meridionalis*	I	B	RH	LITH	INSV		IM	A
Cyprinidae	*Barbus graellsii*	T	B		LITH	OMNI	POTAD	LL	A
Cyprinidae	*Barbus haasi*	I	B	RH	LITH	INSV		IM	A
Cyprinidae	*Carassius auratus*	T	B		PHYT	OMNI		LL	I
Cyprinidae	*Cyprinus carpio*	T	B		PHYT	OMNI		LL	I
Cyprinidae	*Gobio lozanoi*		B	RH		INSV		SL	A
Cyprinidae	*Parachondrostoma miegii*	I	B	RH	LITH			IM	A
Cyprinidae	*Pseudorasbora parva*	T				OMNI		SL	I
Cyprinidae	*Phoxinus bigerri*	I	WC	RH	LITH	OMNI		SL	A

(continued)

Table 2 (continued)

Family	Species	Tolerance	Feeding habitat	Habitat	Reproduction	Feeding group	Migration	Longevity	Status
Cyprinidae	*Rutilus rutilus*	T	WC			OMNI		IM	I
Cyprinidae	*Scardinius erythrophthalmus*	T	WC	LI	PHYT	OMNI		LL	I
Cyprinidae	*Squalius laietanus*		WC	RH	LITH	OMNI		LL	A
Esocidae	*Esox lucius*		WC		PHYT	PISC		LL	I
Gasterosteidae	*Gasterosteus gymnurus*		WC			INSV		SL	A
Gobiidae	*Pomatoschistus microps*		B			INSV	LONG	SL	A
Ictaluridae	*Ameiurus melas*	T	B		LITH	OMNI		IM	I
Mugilidae	*Chelon labrosus*	T					LONG	LL	A
Mugilidae	*Liza ramada*	T					LONG	LL	A
Mugilidae	*Mugil cephalus*	T					LONG	LL	A
Percidae	*Perca fluviatilis*	T	WC			PISC		LL	I
Percidae	*Sander lucioperca*		WC		PHYT	PISC		LL	I
Petromyzontidae	*Petromyzon marinus*	I		RH	LITH		LONG	LL	A
Poeciliidae	*Gambusia holbrooki*	T	WC	LI		INSV		SL	I
Salmonidae	*Oncorhynchus mykiss*			RH	LITH	PISC		IM	I
Salmonidae	*Salmo trutta*	I		RH	LITH	PISC		IM	A
Siluridae	*Silurus glanis*	T	B		PHYT	PISC		LL	I

T tolerant, *I* intolerant, *B* benthic, *WC* water column, *RH* rheophilic, *LI* limnophilic, *LITH* lithophilic, *PHYT* phytophilic, *OMNIV* omnivore, *PISC* piscivore, *INSV* invertivore, *LONG* long migration (diadromous species), *POTAD* short migration, *SL* short longevity, *IM* intermediate longevity, *LL* long longevity, *A* species native from Catalonia, *I* species introduced in Catalonia. Blank means species not classified mean = 1.4), and the number of introduced species (NSI) was from 0 to 10 (median = 0, mean = 0.82).

For selecting candidate metrics, we carefully reviewed the literature including research papers and reports from different countries. In total, for the 311 sites, we computed 199 candidate metrics, which can be classified into four categories as in the original IBI development [51, 66]: species composition and diversity, trophic composition, abundance, and fish condition. All the metrics were in general computed both for native and introduced species separately and for all species together. The native/alien status was considered at the river basin level.

To validate the new indices with gradients of anthropogenic pressure, we used two different anthropogenic disturbance measures. First, we obtained an official statistic of anthropogenic disturbance (the risk of noncompliance measure, RI_AP) from the Catalan Water Agency (document IMPRESS; [67]). It summarizes many different disturbances such as hydromorphological changes, flow regime alterations, changes in land use and the riparian zone, and point and diffuse sources of pollution [47, 67]. Second, a principal component analysis (PCA) was also used to combine this risk of noncompliance with our local measurement at the sampling sites such as the sum of RBI scores, sum of visual impacts, dissolved oxygen concentration, ammonia concentration, and pH. The first PCA axis summarized well a gradient of anthropogenic disturbance (see [47] for details).

The site-specific approach (IBICAT2a) was developed following leading works in Europe [48, 52, 68]. To define the calibration set (low pressure), we followed the usual method (see, e.g., [69, 70]): only sites where none of the pressures (hydrological regime, river connectivity, morphology, toxic acidification, and nutrient organic inputs) was greater than 2, ranging from 1 (no pressure) to 5 (high pressure) were used. Among 369 sites in Catalonia, 49 sites fulfilled all these criteria (of which 34 sites had fish captures). Then, generalized linear models (GLMs), with appropriate error and link functions depending on the types of metrics, were used in the reference condition sites (calibration set) to develop the expected values of fish metrics given numerous natural environmental variables (climatic and topographic) that are not affected by anthropogenic disturbance. A stepwise procedure based on Akaike's information criterion was used to select parsimonious, adequate GLMs. Then the observed values on the rest of sites are compared to the expected values (see, e.g., [71, 72]) to compute an index that ranges from 0 (worst conditions) to 1 (reference conditions).

From the numerous GLMs, we selected 10 metrics considering their significant correlation with anthropogenic disturbance (pressures), their meaningfulness in ecological terms, their complementarity (e.g., different organization levels), and relatively low collinearity. Although the detailed results and a tentative index (IBICAT2a) are given in Sostoa et al. [23], we considered that this index was not suitable because of a number of reasons: (1) the GLMs could not be cross-validated because of low sample sizes and considerable variability in the reference data and probably also because of the considerable environmental heterogeneity of Catalonia; (2) the metrics based on absolute richness and abundance metrics did not behave well (gave unrealistic expected results) probably due to low numbers of reference condition sites (which were mostly at higher elevations) and therefore the index only included relative metrics (i.e., percentages); and (3) dry and fishless sites were not well predicted by predictive models, suggesting many local pressures that are not well captured by available indicators. Therefore, although this approach has been successfully applied in France [48, 52] and across Europe [54, 52, 71] and could potentially be developed in Catalonia, the low sample size available of fish data precludes its current application.

5 IBICAT2b: Development of a Type-Specific Fish Index for Catalonia and the Ebro River Basin

We also attempted a simpler type-specific approach (IBICAT2b), whose results we consider much more reliable than IBICAT2a and that we have validated (through correlation with environmental pressures) throughout Catalonia [23] and the Ebro River (Bae et al. unpublished data). We recommend IBICAT2b as a regional fish index, until further data become available that allow developing a better index. This index uses the official river types based on environmental data that are also used for macroinvertebrate indices and other purposes in Catalonia (e.g., [67, 73, 74]), the whole Ebro River [75], and Spain in general (http://www.chebro.es/; [76, 77]) (Fig. 2, Table 3).

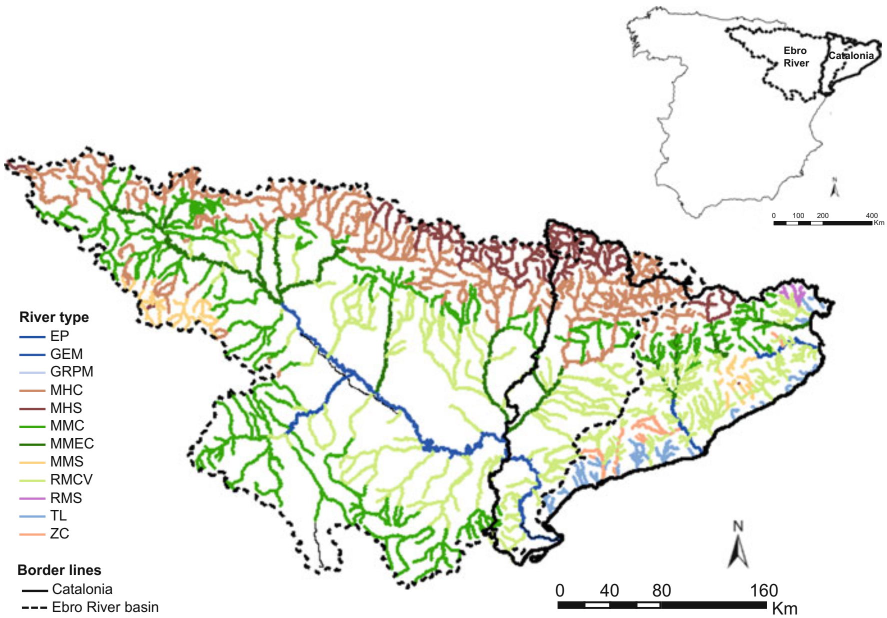

Fig. 2 Official river types in Catalonia and the Ebro River [74, 75]. See Table 3 for the meaning of code abbreviations and further details

Table 3 River typology and number of sites in each river type

Official river type no.	River type	Catalan abbreviation	Number of Catalan sites with fish data used in the study
27	Siliceous wet mountain rivers	MHS	23
26	Calcareous wet mountain rivers	MHC	52
11	Siliceous Mediterranean mountain rivers	MMS	11
12	Calcareous Mediterranean mountain rivers	MMC	47
15	High-flow Mediterranean mountain rivers	MMEC	13
9	Variable-flow Mediterra-nean rivers	RMCV	147
8	Siliceous Mediterranean lowland rivers	RMS	2
10	Rivers influenced by karstic areas	ZC	16
16	Main watercourses	EP	10
18	Coastal streams	TL	32
17	Large Mediterranean watercourses	GEM	6
15	Large rivers with weak mineralization	GRPM	10

In order to select the metrics that reflected well the gradients of anthropogenic disturbance for each river type, we computed the correlations between PC1 (the anthropogenic disturbance described in the previous section) and all the metrics in each typology separately, which is a classical type-specific approach (see [70]). In this procedure, because the total sampling sites in some of the river types were very low (e.g., EP, GEM, GRPM, MMS, and RMS where the total number of sampling sites were less than 11), we used a coarser statistical criteria ($P < 0.1$). In RMS type, we could not calculate correlations because only two sampling sites were available (Table 3). To select the final metrics for the index in each typology, we considered its diversity (different organization levels and type of metrics), complementarity (as assessed with a principal component analysis, which showed different groups of metrics based on their correlation), and interpretability of results (a few metrics had relationships with PC1 opposite than expected). The final metrics selected are shown in Table 4.

These different metrics were scored following a number of approaches. The number of native species was scored based on expert criteria and the historical records of fish assemblages in Catalonia. For DELT anomalies, we used the traditional IBI scoring: 0–2%, very good; 2–5%, moderate; and >5%, bad

Table 4 Metrics selected for the 10 river types. *Metrics in italics* represent metrics that increase with anthropogenic disturbance (and vice versa for metrics not in italics)

River type no.	Catalan abbreviation	Number of sites	Common metrics	Type-specific metrics
27	MHS	23	NSN, *PSI*, *PII*, *PIT_DELT*	PIT_pisciv, PST_pisciv
26	MHC	52	NSN, *PSI*, *PII*, *PIT_DELT*	PIT_pisciv, PST_lithophil, PIT_intol, *PST_SL*
11	MMS	11	NSN, *PSI*, *PII*, *PIT_DELT*	–
12	MMC	47	NSN, *PSI*, *PII*, *PIT_DELT*	NIN_15cmintol
15	MMEC	13	NSN, *PSI*, *PII*, *PIT_DELT*	*PST_SL*
9	RMCV	147	NSN, *PSI*, *PII*, *PIT_DELT*	PIT_intol, NIN_15cmintol, PST_lithophil, PIT_rheophil
8	RMS	2	NSN, *PSI*, *PII*, *PIT_DELT*	–
10	ZC	16	NSN, *PSI*, *PII*, *PIT_DELT*	PST_intol
16	EP	10	NSN, *PSI*, *PII*, *PIT_DELT*	–
18	TL	32	NSN, *PSI*, *PII*, *PIT_DELT*	–
17	GEM	6	NSN, *PSI*, *PII*, *PIT_DELT*	–
15	GRPM	10	NSN, *PSI*, *PII*, *PIT_DELT*	–

NSN number of native species, *PSI* % of exotic species, *PII* % of exotic individuals, *PIT_DELT* % of individuals with deformities, eroded fins, lesions, and tumors (DELT) abnormality, *PIT_pisciv* % of piscivorous individuals, *PST_pisciv* % of piscivorous species, *PST_lithophil* % of lithophilic species, *PIT_intol* % of intolerant individuals, *PST_SL* % of short longevity species, *NIN_15cmintol* native abundance of individuals <15 cm of habitat intolerant species, *PIT_rheophil* % of rheophilic individuals, *PST_intol* % of intolerant species. See Tables 2 and 3 for further abbreviations

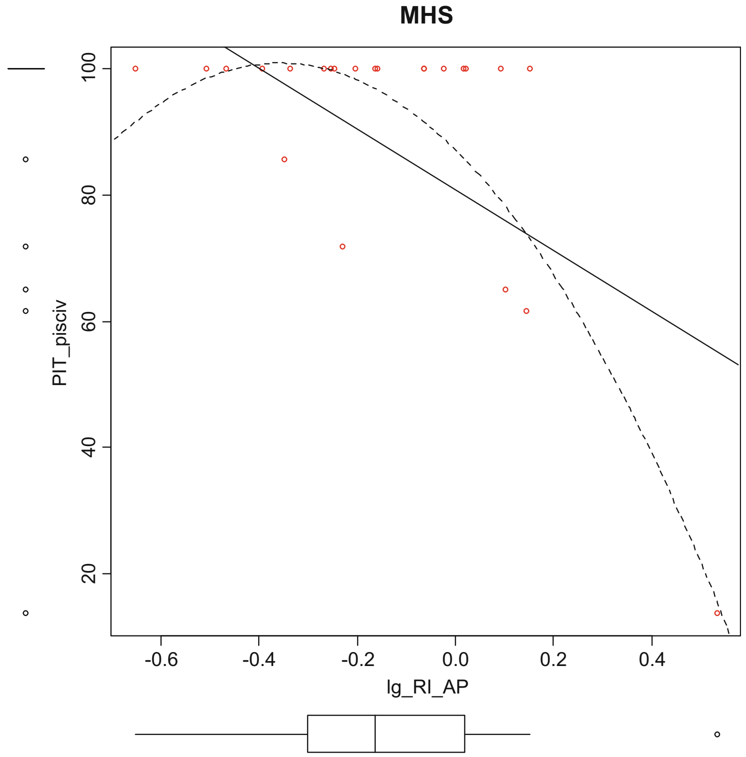

Fig. 3 Relationship between % piscivorous individuals (PIT_pisciv) and anthropogenic pressure (lg_RI_AP: log-transformed RI_AP) in the MHS river type. *Straight line*: linear regression model ($r^2 = 0.375$); *dashed line*: quadratic regression model ($R^2_{adj} = 0.646$). A likelihood ratio test showed that the quadratic model is significantly better than the linear model ($P = 0.0003$)

[41]. For NIN_15cmintol, only presence/absence was considered, because densities were very low and often null despite a clear relationship with anthropogenic disturbance. For the calibration of the other metrics (i.e., PSI, PII, PIT, PIT_pisciv, PST_pisciv, PST_lithophil, PIT_intol, PST_SL, PTI_intol, PST_lithophil, PIT_rheophil, and PST_intol) (see abbreviations in Table 4), the same approach as in the site-specific approach (IBICAT2a) was used for the scoring of metrics. Figure 3 shows the relationship between one of these metrics (PIT_pisciv) and the anthropogenic pressure index in one of the river types (MHS). As shown in this figure, a quadratic model was often significantly better than a linear model. Using these models and the classes defined for the risk of noncompliance measure (RI_AP < 0.8, no risk; 0.8–1.2, low risk; 1.2–2, average risk; >2 high risk) in the IMPRESS official document for Catalonia [67], we predicted PIT_pisciv values corresponding to each threshold and thus obtained the scoring of metrics.

For all the other metrics, we applied the same procedure as with PIT_pisciv to compute the corresponding thresholds based on RI_AP. Finally, the average of the score for relevant metrics depending on river type was computed to obtain the index and the ecological status.

For large rivers (types EP, GEM, GRPM, and MMEC), we also give a "bad" status, if the study reach is dry or no fish was captured after an adequate sampling. There is published [78] and unpublished (personal observations) evidence that Catalan streams are sometimes dry artificially (due to human water abstraction). Conservatively, we only apply this "bad" status classification to large rivers that should be expected to never run dry or be fishless in natural conditions. For other river types, if the sites are dry or no fish was captured, no status is given, because this might be due to natural causes.

Although both indices (IBICAT2a and IBICAT2b) are very different in terms of the development procedure of indices, both indices showed a similar response to anthropogenic disturbance (i.e., the correlation coefficients were 0.41 for IBICAT2a and PC1, −0.36 for IBICAT2a and lg_RI_AP, 0.40 for IBICAT2b and PC1, and −0.33 for IBICAT2b and lg_RI_AP). There was also high correlation between the two indices ($r = 0.71$), although the relationship was nonlinear because many metrics in IBICAT2a often had values of 0 or 1, indicating that IBICAT2a should be revised with more reference sites to develop further the predictive models and underlying index. Even though IBICAT2a showed relatively high correlation with anthropogenic disturbances, it has several limitations (see section above) and should not be used. A map with the results of IBICAT2b in Catalonia is given in p. 120 of Sostoa et al. [23].

6 Protocol for the IBICAT2b Multimetric Fish Index

An Excel file is given as an online supplementary material to this book chapter (http://invasiber.org/EGarcia/IBICAT2b.html) for the computation of the IBICAT2b index in Catalonia and the Ebro River. The index should not be used in other regions unless it is validated for them (i.e., correlated with environmental pressures) and it should be first adapted for different fish faunas. The following steps should be followed to compute the index. They are automated if the data are imputed in the Excel file.

1. Obtain the river type of your sampling reach.

   River types for this index are the general ones official for the WFD across Spain: there are 12 different river types in Catalonia (Table 3) and 8 in the whole Ebro River basin (all of them also present in Catalonia). Note, however, that there is a minor difference between Catalan and Spanish types: type 15 corresponds to two different Catalan types. Furthermore, there are some reaches declared as heavily modified water bodies and without any official type. Find the river type of your sampling reach in Fig. 2.

2. If your sampling sites are in EP, GEM, GRPM, or MMEC river types and they were dry or fishless, ecological status is "bad" (IBICAT2b = 1, EQR = 0). If the sites were dry or fishless but belong to other river types, the status cannot be defined with this index. Otherwise, proceed to point 3.
3. Score each metric with the fish data from the study site.

   All metrics should be independently scored from 1 (bad) to 5 (very good) according to the following tables. Metrics 1–4 are common to all river types. The rest of metrics are for some river types only. If some metrics cannot be computed (e.g., metric 2 has not been measured), they can be omitted from the final average.

   Metric 1: number of native species (NSN)

River type no.	Catalan abbreviation	Very good	Good	Moderate	Poor	Bad
27	MHS	>1		1		0
26	MHC	>1		1		0
11	MMS	>1		1		0
12	MMC	>1		1		0
15	MMEC	>2	2	1		0
9	RMCV	>1		1		0
8	RMS	>1		1		0
10	ZC	>1		1		0
16	EP	>3	3	2	1	0
18	TL	>1		1		0
17	GEM	>4	4	3	2	<2
15	GRPM	>3	3	2	1	0

   Metric 2: percentage of individuals with deformities, eroded fins, lesions and tumors (DELT) abnormality [41]

	Very good	Good	Moderate	Poor	Bad
DELT	0–2%		>2–5%		>5%

   Metric 3: percentage of introduced individuals (PII)

	Very good	Good	Moderate	Poor	Bad
PII	0%		0–5%	5–20%	>20%

   Metric 4: percentage of introduced species (PSI)

	Very good	Good	Moderate	Poor	Bad
PSI	0%		0–5%	5–20%	>20%

   Other metrics: specific metrics for some river types. See Tables 2 and 3 for further abbreviations.

River type no.	Catalan abbreviation	Specific metric	Very good	Good	Moderate	Poor	Bad
27	MHS	PIT_pisciv	100%	99.99–96.67%	96.66–84.80%	84.79–59.84	<59.84%
27	MHS	PST_pisciv	100%	99.99–90.32%	90.31–82.85%	82.84–68.92%	<68.92%
26	MHC	PIT_pisciv	100%	99.99–44.91%	44.90–39%	39–29.46%	<29.46%
26	MHC	PST_lithophil	100%	99.99–97.38%	97.37–95.51%	95.50–91.77%	<91.77%
26	MHC	PIT_intol	100%	99.99–89.27%	89.26–80.22%	80.21–63.34%	<63.34%
26	MHC	PST_SL	0%	0–32.58%	32.58–39.58%	39.58–45.92%	>45.92%
12	MMC	NIN_15cmintol (presence)	Yes		No		
15	MMEC	PST_SL	0%	0–4.14%	4.14–19.95%	19.95–35.73%	>35.73%
9	RMCV	PIT_intol	>65.78%	65.78–56.65%	56.65–47.28%	47.28–0%	0
9	RMCV	NIN_15cmintol (presence)	Yes		No		
9	RMCV	PST_lithophil	100%	99.99–71.55%	71.55–62.79%	62.79–54.32%	<54.32%
9	RMCV	PIT_rheophil	100%	99.99–80.09%	80.09–71.08%	71.08–62.54%	<62.54%
10	ZC	PST_intol	100%	99.99–65.91%	65.91–55.59%	55.59–43.34%	<43.34%

Therefore, IBICAT2b includes 4–8 metrics depending on river type. Each metric is scored from 1 to 5 (1 = bad, 2 = poor, 3 = moderate, 4 = good, and 5 = very good).

4. The final index is computed as the average of all available metrics. To obtain the ecological status according to IBICAT2b, the following thresholds are used:

	Very good	Good	Moderate	Poor	Bad
IBICAT2b	≥4.5	3.5–4.5	2.5–3.5	1.5–2.5	<1.5
EQR	≥0.875	0.875–0.625	0.625–0.375	0.375–0.125	<0.125

7 Concluding Remarks

Another type-specific index ($IBICAT_{2010}$), quite different from IBICAT2b, was also described in Sostoa et al. [23] (see also [4]). An adaptation of this index (IBIMED), so far (February 2015) not available in published papers, Internet reports, or software, was intercalibrated with EFI+ and the Portuguese fish index [79]. The differences between IBIMED and $IBICAT_{2010}$ include the addition of some of the rest of Spanish fish species with their guild classification (to allow the computation in other river basins) [79] and apparently different thresholds for the EQR classes. IBIMED has only been successfully validated with qualitative environmental pressures in Mediterranean rivers and the Duero and not the rest of Spanish rivers and was only intercalibrated for Mediterranean rivers (excluding the Duero) [79]. Recent unpublished work throughout the Ebro River (García-Berthou and Bae, unpublished data) shows that IBICAT2b and EFI+ are more related to quantitative environmental pressures than IBIMED/$IBICAT_{2010}$, which shows problems mainly in the typology and treatment of fishless or dry sites. However, these three indices are correlated and their values could thus be converted (e.g., $IBICAT_{2010} = 0.2099 + 0.1398$ IBICAT2b, IBICAT2b $= 1.3849 + 2.941$ $IBICAT_{2010}$, $r^2 = 0.411$, $P < 0.0005$; EFI+ $= 0.2686 + 0.1279$ IBICAT2b, IBICAT2b $= 1.8573 + 2.2129$ EFI+, $r^2 = 0.283$, $P < 0.0005$). Overall, our work suggests that fish indices can be successful in Spain but research is needed to improve them and generalize them. The availability of further fish data, user-friendly software, and extensive validation are essential steps toward the improvement of these fish-based indices.

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Biological Indicators to Assess the Ecological Status of River-Dominated Estuaries: The Case of Benthic Indicators in the Ebro River Estuary

Carles Ibáñez, Nuno Caiola, Rosa Trobajo, Alfonso Nebra, and Laia Rovira

Abstract River-dominated estuaries (also known as salt-wedge or highly stratified estuaries) are transitional water bodies occurring in micro-tidal coasts such as the Mediterranean. Their hydrological and ecological particularities make difficult the assessment of the ecological status using either the procedures for rivers or estuaries. For instance, river-dominated estuaries become rivers when the discharge is higher than its annual average (riverine conditions), whereas they become highly stratified when discharge is lower than its annual average (estuarine conditions). Moreover, the transition between riverine and estuarine conditions is abrupt and irregular across space and time, converting these transitional water bodies in naturally stressed ecosystems. To add more complexity, the human intervention in river basins (i.e. damming and intensive water use) has tended to reduce and homogenise river discharge, making more frequent and regular the presence of a salt wedge in the estuary, softening their natural stressful dynamics. As a result, it is difficult to discern natural from anthropogenic stressors, because the increase in environmental stability leads to higher complexity in biological communities and thus some bioindicators may show scores indicating better ecological status under impacted conditions than under natural conditions, which is an expression of a phenomenon known as ‘estuarine quality paradox’. To sort out this situation and achieve a proper assessment of the ecological status of river-dominated estuaries, a specific approach is required, both in terms of the bioindicators to be used and the methodology to make them work in the correct way.

In this chapter a synthesis of preliminary work carried out to develop assessment methods (according to the Water Framework Directive) in the Ebro River estuary is presented, and the strategy to further develop the best methods to carry out the ecological status assessment is discussed. The Ebro River estuary is a typical salt-

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wedge estuary which is representative of this type of water bodies in the Mediterranean, and its hydrology and ecology have been intensively investigated in the past. Results show that existing bioassessment methods for transitional waters are not appropriate for the assessment of the ecological status of river-dominated estuaries, though in some cases the adaptation of some methods can be a useful way to start with the assessment as long as limitations are known.

Keywords Diatoms, Ecological indicators, Macroinvertebrates, Salt-wedge estuary

Contents

1 Introduction

The European Union reacted to the severe ecological decline of aquatic ecosystems by passing the Water Framework Directive (WFD) in 2000 [1]. The WFD provides a basis for the conservation, protection and improvement the ecological integrity of all water bodies, including groundwater, inland surface water and coastal and transitional waters. According to the WFD, the estuaries are classified as transitional waters, defining them as bodies of surface water in the vicinity of river mouths which are partly saline in character as a result of their proximity to coastal waters but which are substantially influenced by freshwater flows. The WFD aims to assess the ecological status of all European water bodies using hydromorphological, physicochemical and biological indicators (i.e. phytoplankton, macroalgae, phytobenthos, macroinvertebrates and fish) [2, 3]. Ecological quality assessment of a water body must be based on the status of different biological quality elements (e.g. benthic invertebrate fauna or aquatic flora) and endorsed by hydromorphological and physicochemical quality elements. The status of these elements is determined by the deviation they exhibit from the type-specific reference conditions, at undisturbed or nearly undisturbed situations (WFD, 2000/60/EC, Annex V).

Estuaries are dynamic ecosystems showing a high spatial and temporal physico-chemical and biological variability, and they can also present several pollution gradients due to the high number of human activities influencing them [4, 5]. Moreover, in transitional waters natural stressors interact with anthropogenic stressors, making it very difficult to discern between them in terms of impacts on the biological communities, because the increase in environmental stability leads to higher complexity in biological communities and thus some bioindicators may show scores indicating better ecological status under impacted conditions than under natural conditions, which is an expression of a phenomenon known as ‘estuarine quality paradox’ [6, 7]. The rapid population growth during the last century has increased the pressures over estuarine systems, threatening their ecological integrity, economic value and even affecting public health [6–9]. The main anthropogenic pressures affecting estuaries are industrial waste water, urban sewage effluents, agriculture and farmland runoff, fish farming and harbours [10]. These activities cause an excess of nutrients, increase the organic matter loads and even promote the accumulation of dangerous pollutants in the sediment such as heavy metals, toxic compounds and hydrocarbon substances [11, 12]. High nutrient loads produce direct ecological impacts over biological communities [13], associated mostly with eutrophication processes [14]. These facts disturb composition, trophic structure and biomass of the biological communities [15, 16].

In Mediterranean aquatic ecosystems, the impacts produced by these pressures are magnified by the strong seasonal and inter-annual hydrological variability [17, 18]. Moreover, human responses to this hydrological fluctuation involve flow regulation measures, such as reservoirs, that frequently disrupt aquatic ecosystems, producing accentuated environmental changes [19]. In highly stratified estuaries, like the study case, obtaining a coherent response of biotic indices to abiotic stressors is even more difficult because both natural and anthropogenic hydrological variations (spatial and temporal) produce rapid and abrupt changes in biological communities [20]. Therefore, establishing reference conditions for these systems (the basis for the development of biotic indices according to the WFD criteria) is a challenging task.

In this chapter a synthesis of preliminary work carried out to develop assessment methods (according to the Water Framework Directive) in the Ebro River estuary (southern Catalonia) is presented (see [20, 21] for more information), and the strategy to further develop the best methods to carry out the ecological status assessment is discussed. The Ebro River estuary is a typical salt-wedge estuary which is representative of this type of water bodies in the Mediterranean, and its hydrology and ecology have been intensively investigated.

2 Benthic Bioindicators to Assess the Ecological Status of Water Bodies

The most widespread benthic bioindicators used both in the European Union and elsewhere to assess the ecological status of water bodies are benthic diatoms and macroinvertebrates. Due to its reduced mobility and short generation times, phytobenthos has shown a rapid response to environmental changes and can integrate environmental conditions better than other bioindicators [22], being commonly used in the assessment of the ecological status and monitoring of anthropogenic impacts. Diatoms are the main component of phytobenthos and are one of the most important groups of algae used for ecological assessment [23–26]. Their ubiquity, their direct and sensitive response to physicochemical changes and their preservation in sediments for a long time make them good water quality indicators for both present and past environmental changes [22]. In Europe there are about 20 diatom-based metrics that were initially developed to assess nutrient and/or organic pollution in rivers, and, later, some of them have been adapted to fulfil the WFD requirements of assessing the ecological status of these ecosystems [27]. However, little information is available about the use of benthic diatoms as bioindicators in estuaries and other transitional systems, with only very few studies carried out in Europe [28, 29] and in the USA [30]. The study of Della Bella et al. [28] is the only one dealing with the controversies of water quality assessment in these complex water bodies. And certainly there is no diatom index specific for transitional or marine waters.

Benthic invertebrates also play important roles in the ecology of aquatic ecosystems and respond to anthropogenic stress [9, 15, 31–33]. During the last decade, some biotic indices based on soft-bottom benthic invertebrate communities such as the AMBI [34], BENTIX [32] and the multivariate method M-AMBI [35, 36] have proved to be very useful tools in assessing the ES of coastal and TWs, especially regarding nutrient and organic enrichment. However, the estuarine systems where these indices were developed correspond to ‘well-mixed’ type, which are systems with different ecological dynamics comparing with ‘highly stratified’ estuaries like the Ebro estuary.

3 Study Area and Methods

The Ebro estuary (Fig. 1) is a salt-wedge estuary located in southern Catalonia, at the NE of the Iberian Peninsula (40°43′10″N, 0°40′30″E); it covers an approximate area of 10 km^2 and is 40 km long with a mean width of 237 m and a mean depth of 6.8 m. It is a micro-tidal estuary with a tidal range around 20 cm, favouring the vertical stratification of the water column and the existence of a salt wedge, with a maximum intrusion in the Ebro River of 32 km. The hydrology and dynamics of the salt wedge is controlled mainly by the river flow, as other salt-wedge estuaries

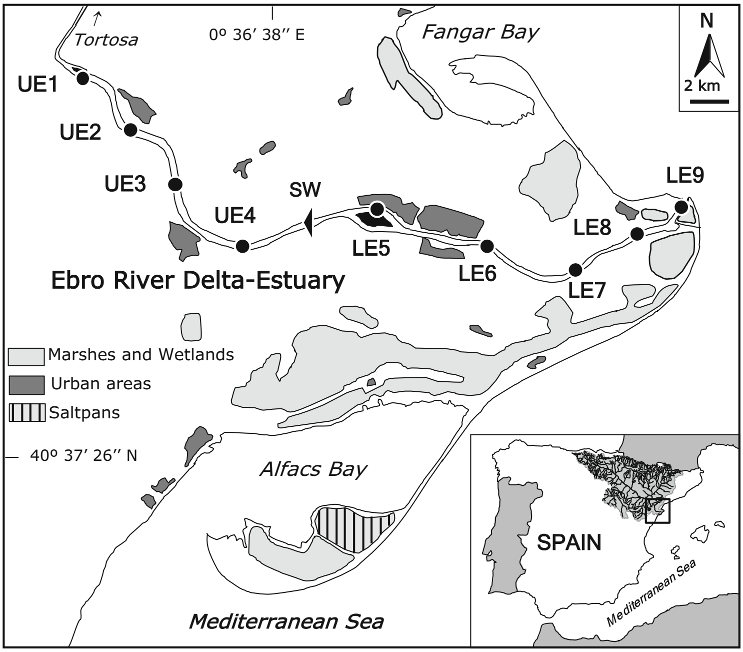

Fig. 1 Map of the Ebro River basin and its delta showing the studied estuary with the position of the nine sampling stations. *UE* upper estuary stations, *LE* lower estuary stations, *SW* null point position

[37]. When the Ebro River flow is above 400 m^3/s, the wedge is absent; between 300 and 400 m^3/s, it occupies the last 5 km of the estuary, whereas with discharges below 300 m^3/s, it advances up to 18 km from the river mouth (this is the most frequent situation); when the flow is less than 100 m^3/s, the wedge reaches its maximum extent.

The lower Ebro River flow has been largely regulated since 1960s with two big reservoirs (Mequinenza and Riba-Roja) situated 100 km upstream the river mouth, and it has decreased by 40% due to intensive water uses in the Ebro basin, with irrigation accounting for 90% of water consumed [38]. The main human impacts at basin level are the hydrological alteration resulting from strong flow regulation and water abstraction, and the high nutrient levels in river water due to the input of agricultural and urban sewage effluents [39–41]. Nevertheless, during the last 15 years, an improvement of urban sewage treatment together with the restriction in the use of phosphate-based compounds has dimmed the eutrophication process [42–44].

3.1 Benthic Samples

For benthic diatoms, eight sampling sites distributed every 3–6 km within the estuary were sampled every 3 months from October 2007 to December 2008 (see [21, 45] for details). Benthic diatom samples were collected from both natural and artificial substrata (fired clay bricks). An area of 4 cm^2 was scrapped off the artificial substrata, and three fragments from natural substrata were included in each replicate. Two replicates from both artificial and natural substrata were processed. Benthic diatom samples were oxidised with H_2O_2 30% v/v a few hours in order to remove the organic matter, and HCl^- 37% v/v was added to eliminate carbonates; clean valves were permanently mounted with Naphrax©. Slides were examined using a LEICA DMI 3000B light microscope equipped with differential interference contrast under oil immersion objective at x100 magnification. A minimum of 400 valves were counted at both natural and artificial replicates, and identification of diatoms was done down to species level using specialised bibliography [46–48]. A total of nine sites were sampled for benthic macroinvertebrates (the same sampling stations sampled for benthic diatoms plus one extra site). Each station was sampled seasonally (summer and autumn 2007; winter and spring 2008) for benthic macroinvertebrates, sediment traits, dissolved oxygen, total and dissolved nutrients and hydromorphological characteristics (depth, flow velocity and water transparency, suspended sediment and chlorophyll *a*) (see [20] for details on sample and laboratory procedures). These abiotic parameters were also recorded for benthic diatoms surveys.

3.2 Biotic Indices and Metrics Evaluation

A screening of existing benthic diatoms and macroinvertebrates biotic indices for the assessment of surface waters' ecological status was carried out. All the indices that could potentially be suitable to assess the ecological status of the Ebro estuary were computed (Table 1), and their response to anthropogenic disturbances was tested. Moreover, this analysis was performed for three groups of diatom indicator species identified for the three main ecological conditions recognised for the Ebro estuary [21, 45], i.e. riverine conditions, estuarine conditions and well-established salt-wedge conditions (Table 2). These diatom indicator species were identified through Indicator Species Analysis [68]; for further details, see [21]. In the case of macroinvertebrate multimetric indices, the individual metrics responses to human disturbance were also assessed.

The anthropogenic disturbance was expressed with three variables: a pollution index and two variables defining the hydrological alteration [67]. In the case of diatoms, the pollution index was expressed as a nutrient gradient that corresponds to the significant factor resulted from a principal component analysis performed with the water nutrients (P-PO_4^{-3}, N-NO_3^-, N-NO_2^-, N-NH_4^+). For

Table 1 List of the 17 diatom indices (developed for rivers) and 4 macroinvertebrate indices (developed for river or estuaries) that have evaluated in this study

Code	Index	Source
CEE	Descy and Coste Diatom Index	Descy and Coste [23]
DESCY	Descy Index	Descy [49]
DI-CH	Swiss Diatom Index	Buwal [50]
EPI-D	Diatom-Based Eutrophication/Pollution Index	Dell'Uomo [51]
GENRE	Generic Diatom Index	Rumeau and Coste [52]
IBD	Biological Diatom Index	Lenoir and Coste [53]
IDAP	Artois-Picardie Diatom Index	Prygiel et al. [54]
IDP	The Pampean Diatom Index	Gómez and Licursi [55]
IPS	Specific Pollution Sensitivity Index	Cemagref [56]
L&M	Leclerq and Maquet Index	Leclercq and Maquet [57]
LOBO	LOBO Index	Lobo et al. [58]
SHE	Schiefele and Schreiner Index	Schiefele and Schreiner [59]
SID	Austrian Saprobic Index	Rott et al. [60]
SLA	Sládecěk Index	Sládeček [61]
TDI	Trophic Diatom Index	Kelly [62]
TID	Austrian Trophic Index	Rott et al. [63]
WHAT	Watanabe Index	Watanabe et al. [64]
IBMWP	Iberian Biological Monitoring Working Party	Alba-Tercedor et al. [65]
M-AMBI	Multivariate Marine Biotic Index	Borja et al. [3], Muxika et al. [36]
BENTIX	BENTIX	Simboura and Zenetos (2002) [32]
BOPA	Benthic Opportunistic Polychaetes Amphipods Index	Dauvin and Ruellet [66]

Code: index abbreviation; source: publication from which the index was first described. See references in [21] and [67]

macroinvertebrates, the pollution index was expressed as an organic pollution index which is a synthetic value of the two first factors from a principal component analysis performed with the organic pollution-related variables (DO, nutrients, chlorophyll a, pheophytin and organic matter in sediment and in suspension). The hydrological alteration was expressed as the deviation of the salt-wedge dynamics from the expected natural condition in both probability and time of presence in each sampling occasion [67].

The criterion used to evaluate the performance of the different diatom and macroinvertebrate metrics in assessing the ecological status of the Ebro estuary was based on the existence of a significant correlation with the pressure variable (i.e. pollution or hydrological alteration) and in the expected response to increasing perturbation. The benthic diatoms and macroinvertebrates of the Ebro estuary are structured in two communities associated with the upper (UE) and lower estuary (LE) stretches and independent from the sampling season [20, 45] (in the latter, UE

Table 2 Diatom indicator species list for each of the three main ecological conditions recognised for the Ebro estuary by Rovira et al. [21]

	IV	S	F
Riverine conditions			
Cocconeis placentula var. *trilineata*	85	88	97
Cocconeis placentula var. *euglypta*	84	84	100
Amphora pediculus	80	80	100
Navicula antonii	80	84	95
Navicula cryptotenella	78	80	98
Amphora cf. *vetula*	78	89	88
Achnanthidium minutissimum	77	91	84
Navicula cf. *cryptotenelloides*	63	83	76
Nitzschia amphibia	60	79	76
Estuarine conditions			
Nitzschia inconspicua	83	90	92
Amphora polita	69	97	71
Navicula aff. *mollis*	69	82	84
Tabularia fasciculata	65	73	89
Navicula recens	61	65	95
Navicula gregaria	61	89	68
Nitzschia constricta	61	93	66
Navicula perminuta	60	95	63
Well-established salt-wedge conditions			
Diploneis sp.	56	96	58
Amphora aff. *luciae*	34	99	34
Gomphonemopsis obscura	23	96	24
Cocconeis cf. *neothumensis* var. *marina*	18	100	18
Parlibellus cf. *berkeleyi*	18	100	18
Planothidium iberense	16	100	16

IV indicator value, *S* specificity, *F* fidelity

and LE mainly correspond to ‘riverine’ and ‘estuarine’ conditions, respectively). Therefore, the sensitivity of the biotic indices and metrics to human disturbance was analysed separately for the two stretches, and seasonality was not taken into account [21, 67].

4 Results and Discussion

4.1 Application of Benthic Diatom Indices

There are no specific indices for assessing ecological status of estuaries and other transitional waters using diatoms. Therefore, as a first step towards developing such

Table 3 Significant Spearman coefficients for the upper estuary (UE) between the tested metrics and the nutrient gradient (PCA axis 1) and hydrological pressure (expressed as the deviation of the probability of the salt-wedge occurrence over a month from the estimated probability under natural flow condition for that month)

	Nutrient gradient	P_SaltWedge
IPS	0.581**	-0.396*
SLA		
DESCY	0.413*	
LMA		
GENRE		
CEE		
SHE		
WHAT	0.375*	-0.457*
IDAP	0.393*	
IBD		
DI-C		
EPI-D		
IDP	0.551**	
LOBO		
SID		
TID		
TDI	-0.516**	
∑ RA of riverine indicator species	-0.386*	
∑ RA of estuarine indicator species		
∑ RA of well-established salt-wedge indicator species		

RA relative abundances
*$p < 0.05$, **$p < 0.01$

an index, we evaluated the application of 17 diatom-based indices developed for rivers (see [21]) to the Ebro estuary. The ecological status classification of the Ebro Estuary depended entirely on which index was applied. For any given sampling campaign, different indices showed very different status class assessments of the Ebro estuary (see Table 4 in [21]). In general, samples of estuarine conditions (most samples of LE) showed lower ecological status values than samples of riverine conditions (most samples of UP). For some indices, these differences resulted in an inferior ecological status class. However, and more importantly, all indices showed a strong and negative correlation with salinity, but none were strongly and negatively correlated with nutrients, except the TDI; this showed a strong correlation with nutrients, which (as expected for any such index of nutrient status) was negative (Tables 3, 4 and 6 in [21]). In order to remove any possible effect of salinity on the relationship between indices and nutrient enrichment, Rovira et al. [21] analysed subsets of samples with more stable conductivity. They showed that the negative correlation between indices and conductivity was still very strong in the case of upstream superficial sites, and again only a very few indices showed negative responses to nutrients. Correlation between conductivity and indices in

Table 4 Significant Spearman coefficients for the lower estuary (LE) between the tested metrics and the nutrient gradient (PCA axis 1) and hydrological pressure (expressed as the deviation of the salt-wedge presence – expressed as probability and duration – from the monthly average probability and duration in days during natural flow periods)

	Nutrient gradient	P_SaltWedge	Ndays_SaltWedge
IPS			-0.473**
SLA			
DESCY			-0.411*
LMA			-0.535**
GENRE			
CEE			-0.461**
SHE			
WHAT			-0.510**
IDAP			-0.429*
IBD			-0.441**
DI-C	0.510**		
EPI-D			-0.372*
IDP	0.446**		
LOBO			
SID			
TID			-0.511**
TDI			
∑ RA of riverine indicator species			-0.553**
∑ RA of estuarine indicator species			0.365*
∑ RA of well-established salt-wedge indicator species	0.351*	0.435**	0.413*

RA relative abundances
*$p < 0.05$, **$p < 0.01$

salt-wedge samples did not show a clear pattern, being positively or negatively correlated depending on the index considered. Negative correlations between diatom-based indices and nutrient concentrations increased when salt-wedge samples were considered alone.

Interestingly in the LE, Spearman coefficients showed a strong significant correlation between the variable 'hydrological pressure' and some diatom species indicators of the three main ecological conditions in the Ebro estuary (Table 4). Therefore, for the LE, the following three metrics, ∑ relative abundances (RA) of riverine indicators species, ∑ RA of estuarine indicators species and ∑ RA of well-established salt-wedge indicator species (for the list of indicator species, see Table 2), could help monitor the hydrological alteration of the Ebro estuary. Thus, in the LE, high abundances of estuarine and salt-wedge indicator species would indicate a hydrological alteration due to flow reduction at times when riverine conditions (i.e. the absence of a salt wedge) would be expected (e.g. in spring due to strong rainfall and meltwater). On the other hand, at times of the year when a salt wedge would be expected to be present, hydrological alteration in the estuary could be revealed by high abundances of riverine indicator species.

4.2 Application of Benthic Macroinvertebrate Indices

Spearman correlations between the analysed indices plus single metrics and anthropogenic pressures for the UE and LE are shown in Tables 5 and 6, respectively.

IBMWP: all the families found in UE stations computed for IBMWP calculation. Concerning UE stations, 12.4% were classified as 'Good', 18.8% as 'Moderate', 31.3% as 'Poor' and 37.5% as 'Bad'. There wasn't any station achieving 'High' ES. The worst ES ratings corresponded to stations UE3 and UE4 which ranged between 'Bad' and 'Poor' (Fig. 2); UE2 ranged between 'Moderate' and 'Good' achieving this category in summer and spring. Station UE1 ratings ranged from 'Bad' to 'Moderate'. However, Spearman correlation coefficients reported no significant correlations between IBMWP (and its individual metrics) and the analysed variables concerning hydrological pressure and organic pollution pressure (Tables 5 and 6).

M-AMBI: the percentage of non-scoring taxa in LE stations was very low (0.14% $\pm$ 0.30). Results showed that 25.00% of LE stations were classified as 'High', 45.00% as 'Good', 15.00% as 'Moderate' and 15.00% as 'Poor'; there were no 'Bad' ES ratings (Fig. 2). Spearman correlation reported some significant correlations (in the LE) between some metrics of M-AMBI and the analysed hydrological pressures, but not for the organic pollution pressures (Tables 5 and 6).

BENTIX: similarly to M-AMBI, BENTIX index showed similar percentages of non-scoring taxa 0.18% $\pm$ 0.30. Within LE stretch, the 25.00% of stations were classified as 'High', 5.00% as 'Good', 55.00% as 'Moderate' and 15.00% as 'Poor'; there were no 'Bad' ES ratings. Contrary to M-AMBI, best ES ratings corresponded to LE5 which ranged between 'Moderate' and 'High' (Fig. 2). Spearman correlation coefficients reported significant correlations (in the LE) between some metrics of BENTIX and the analysed hydrological pressures, but not for the organic pollution pressures (Tables 5 and 6).

BOPA: according to this index, the benthic estuarine condition ranged between 'High' and 'Poor' ES categories; there were no 'Bad' ES rating. A 45.00% of LE stations were classified as 'High', 25.00% as 'Good', 20.00% as 'Moderate' and 10.00% as 'Poor'. Spearman correlation coefficients reported significant correlations (in the LE) between some metrics of BOPA and the analysed hydrological pressures, but not for the organic pollution pressures (Tables 5 and 6).

Fig. 2 Ecological status classification of UE and LE stations recorded at each sampling occasion after applying the four different macroinvertebrate BIs: IBMWP, M-AMBI, BENTIX and BOPA. See Fig. 1 for sampling stations' codification

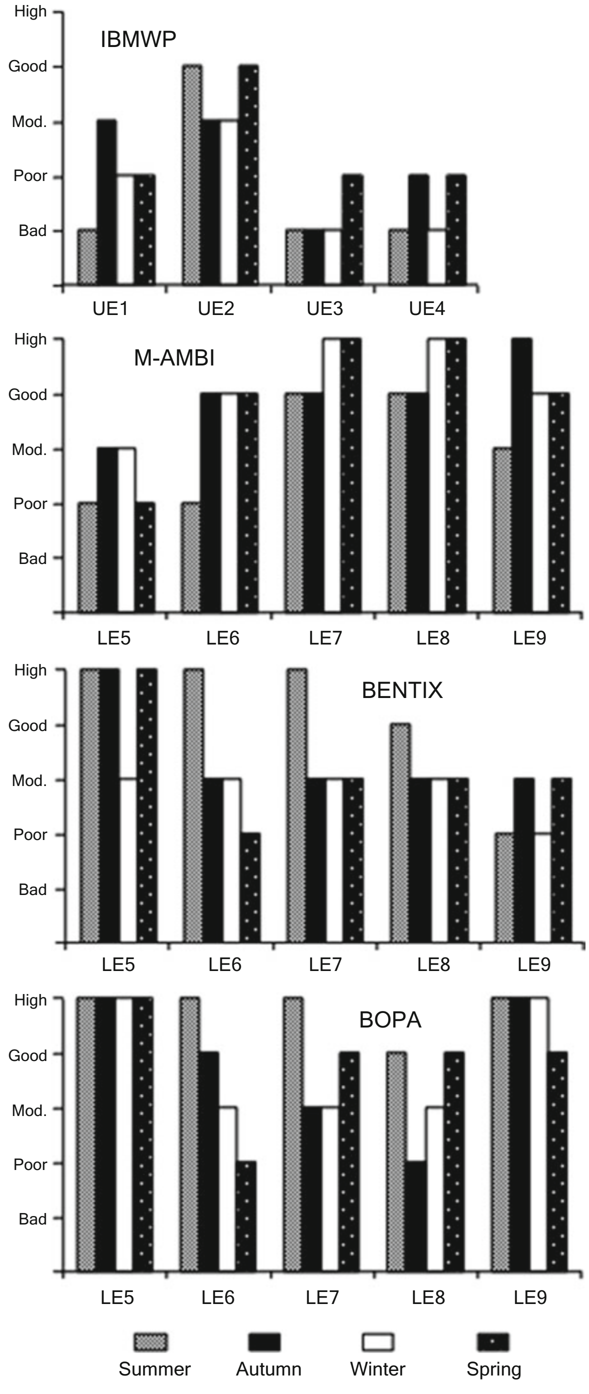

Table 5 Spearman correlations between macroinvertebrate metrics and anthropogenic pressures for the upper Ebro estuary (UE). Correlations having the expected response to pressures are indicated with * if $p<0.05$ or ** if $p<0.01$. Metrics highlighted in grey are the ones potentially useful to develop biotic indicators

	Hydrological alteration (P)		Organic pollution	
Metrics code	Metric	Transformed metric	Metric	Transformed metric
S	−0.3814	−0.4183	0.0921	0.1319
N	−0.2656	−0.4298	0.3921	0.4356
Density	−0.2656	−0.4305	0.3921	0.4357
d	−0.3019	−0.336	−0.0262	0.0093
J	0.2685	0.2242	−0.5628	−0.5844
H	−0.0812	−0.1103	−0.4499	−0.5046
1-l’	0.0418	0.0208	−0.555	−0.5759
DF_%	−0.3117	−0.4836	0.1862	0.2321
G_%	−0.3223	−0.3007	0.391	0.4018
O_%	0.7622**	0.6281**	−0.2119	−0.1865
Pa_%	−0.3232	−0.364	−0.3363	−0.2972
Pr_%	−0.3466	−0.3789	0.0827	0.203
SF_%	0.4388	0.3259	−0.2886	−0.2852
BENTIX[1]	0.1748	0.1041	0.4074	0.3301
Bentix_1_%	0.2269	0.2477	0.463	0.4568
Bentix_2_%	0.4279	0.3584	−0.5178	−0.6029
Bentix_3_%	−0.3885	−0.4844	−0.0436	−0.0609
BOPA[2]	–	–	–	–
BOPA_Amphipod	0.3795	0.5052	0.3823	0.2784
BOPA_Polych	–	–	–	–
AMBI[3]	−0.6134	−0.6295	0	−0.0157
M_AMBI	0.27	0.2757	−0.2531	−0.2455
AMBI_1_%	0.096	0.064	0.1629	0.1556
AMBI_2_%	−0.142	−0.1604	−0.1315	−0.0275
AMBI_3_%	0.6356**	0.5708*	−0.0082	−0.0312
AMBI_4_%	−0.2981	−0.3995	0.1232	0.0914
AMBI_5_%	−0.5928	−0.6416	−0.0143	−0.0389
IBMWP	−0.3354	−0.3874	0.0089	0.1128
EPT_Taxa	−0.3234	−0.3858	−0.0757	−0.1869
EPT_Taxa_%	0.1259	0.0267	−0.445	−0.4955
EPT_Taxa_%_AT	−0.0955	−0.2291	−0.3302	−0.3777
EPT/OL	−0.4028	−0.4844	−0.029	−0.1296
EPT/OL_%	−0.1842	−0.2751	−0.2312	−0.2621
EPT/Diptera	−0.3482	−0.412	−0.1152	−0.1073
EP_Taxa	−0.3549	−0.3835	−0.2969	−0.3207
EP_%	0.2139	0.1306	−0.4184	−0.5003
EP/Tot_%	0.2139	0.1306	−0.4184	−0.5003
OD_Taxa_%	−0.2267	−0.4129	0.2128	0.2614
EPTCBO_Taxa	−0.3991	−0.471	−0.1225	−0.2785
Families	−0.4583	−0.5247*	0.0088	0.0386
Genera	−0.4342	−0.487	0.014	0.0258

Table 6 Spearman correlations between macroinvertebrate metrics and anthropogenic pressures for the lower Ebro estuary (LE). Correlations having the expected response to pressures are indicated with * if $p<0.05$ or ** if $p<0.01$. Metrics highlighted in grey are the ones potentially useful to develop biotic indicators

	Hydrological alteration (P)		Hydrological alteration (n° days)		Organic pollution	
Metrics code	Metric	Transformed metric	Metric	Transformed metric	Metric	Transformed metric
S	0.4094	0.4035	0.7392	0.7927	0.0356	0.0432
N	0.13	0.1881	0.2438	0.2882	−0.2629	−0.217
Density	0.13	0.1882	0.2438	0.2882	−0.2629	−0.2168
d	0.4526	0.4292	0.8382	0.8597	0.1259	0.1162
J	0.3226	0.3271	0.3901	0.393	0.3343	0.3329
H	0.4649	0.4381	0.6531	0.629	0.3508	0.3152
1-l’	0.3924	0.3817	0.5064	0.4999	0.3468	0.3321
DF_%	0.2861	0.3177	−0.1463	−0.1032	0.2773	0.2948
G_%	−0.1461	−0.0551	−0.1089	−0.0057	−0.3393	−0.3001
O_%	−0.0907	−0.0738	−0.344	−0.3325	0.1952	0.2034
Pa_%	0.23	0.3528	0.5295*	0.656**	−0.0729	0.0017
Pr_%	0.4379	0.4024	0.6287	0.6745	0.335	0.2395
SF_%	−0.6311**	−0.6134*	−0.2826	−0.2819	−0.5391*	−0.5353*
BENTIX[1]	−0.3348	−0.3289	−0.6498**	−0.618**	−0.0632	−0.058
Bentix_1_%	−0.3325	−0.2924	−0.6495**	−0.5526*	−0.0631	−0.0352
Bentix_2_%	−0.0514	0.0384	0.459	0.5722*	−0.1561	−0.0965
Bentix_3_%	0.5492*	0.5954*	0.1682	0.2582	0.3453	0.3624
$BOPA_2$	0.4415	0.4446	0.473	0.4821	0.1851	0.1929
BOPA_Amphipod	−0.3258	−0.2695	−0.6466**	−0.5955*	−0.0488	0.0164
BOPA_Polych	0.4332	0.4652	0.4425	0.5176*	0.1726	0.2283
AMBI[3]	0.3331	0.3302	0.2735	0.2705	0.0397	0.0223
M_AMBI	0.4631	0.4619	0.7806	0.7883	0.2139	0.2032
AMBI_1_%	0.1124	0.1334	0.0048	0.0398	0.1136	0.1497

AMBI_2_%	0.1369	0.1967	0.5784	0.6673	0.2314	0.2183
AMBI_3_%	−0.3558	−0.3428	−0.4309	−0.3962	−0.194	−0.1454
AMBI_4_%	0.3219	0.3753	0.4622	0.5604*	0.1367	0.1953
AMBI_5_%	0.4574	0.5884*	−0.0832	0.017	0.1461	0.299
IBMWP	0.4126	0.4144	0.0506	0.0178	0.1512	0.2565
EPT_Taxa	0.1391	0.1391	−0.1554	−0.1554	−0.2153	−0.2153
EPT_Taxa_%	0.3588	0.3263	−0.073	−0.0692	−0.154	−0.1806
EPT_Taxa_%_AT	0.1741	0.15	−0.1878	−0.1682	−0.2018	−0.212
EPT/OL	–	–	–	–	–	–
EPT/OL_%	–	–	–	–	–	–
EPT/Diptera	0.2757	0.2515	−0.0643	−0.0822	−0.2008	−0.205
EP_Taxa	0.1391	0.1391	−0.1554	−0.1554	−0.2153	−0.2153
EP_%	0.3588	0.3046	−0.073	−0.0878	−0.154	−0.186
EP/Tot_%	0.3588	0.3046	−0.073	−0.0878	−0.154	−0.186
OD_Taxa_%	0.111	0.2135	−0.1294	−0.0364	0.1592	0.2057
EPTCBO_Taxa	0.2523	0.2523	0.0339	0.0339	−0.0389	−0.0389
Families	0.541	0.5818	0.3601	0.3387	0.3005	0.346
Genera	0.4755	0.5318	0.3031	0.2968	0.2712	0.3227

4.3 Anthropogenic Pressures Affecting the Ebro Estuary

Results showed that, at present, the main anthropogenic pressure in the Ebro estuary is associated with the hydrological alteration of the lower Ebro River (i.e. increased salt-wedge presence and river flow stability) and that some biotic indices or some of their individual metrics respond to the anthropogenic pressures, especially to the hydrological ones for the lower estuary (showing a higher frequency of salt wedge). Both salt-wedge presence and periods of low and stable flows are natural processes occurring in a stratified estuary with scarce and seasonal rainfall periods. However, increased irrigation and reservoir construction in early 1960s caused a decrease of 40% of the lower Ebro River flow [39, 69, 70], and therefore flow is lower and more stable now than before intensive water use. Flow regulation increased the presence of the salt wedge during most part of the year and reduced changes in its position [37, 69], causing a potential impact on biological communities, not only at a spatial scale, because the salt wedge is found further upstream than before reservoir construction, but also at a temporal scale, because the salt wedge is sometimes now present during meltwater and rainfall periods.

Regarding nutrient concentrations, the lower Ebro River and its estuary showed severe eutrophication due to phosphorus enrichment during the 1980s and 1990s. This situation changed since 1995–1996, when phosphorus concentration suddenly decreased from values of 0.2–0.3 mg/L P-$PO_3{}^{-4}$ to values of 0.05 mg/L P-$PO_3{}^{-4}$ of nowadays [20, 21, 42, 43]. This decrease in phosphorus could be explained by the construction in mid-1990s of waste water treatment facilities in the main cities of the middle Ebro basin together with the banning of detergents with phosphates; these may have reduced eutrophic conditions and therefore decreased phytoplankton concentrations [42, 43, 71]. However, the same trend has not been observed for nitrate concentration, likely due to its origin from non-point sources from agriculture, which are much more difficult to control [71]. Nowadays, nutrient concentrations in the lower Ebro River and more specifically in its estuary (i.e. the last 40 km) are relatively low and show low seasonal variability when compared to other large Mediterranean rivers [72–74], as well as low spatial variability. Therefore, it seems that at present, nutrient enrichment may not constitute the main anthropogenic pressure in the Ebro estuary. Moreover, the analysed biotic indices and their individual metrics do not respond significantly to the organic pollution pressure.

4.4 WFD: Water Quality vs. Ecological Status and the Complexity in Assessing the Latter in TWs

Most of the existing diatom and macroinvertebrate indices were originally designed to assess nutrient and organic pollution in water bodies in response to the requirements of the Urban Wastewater Treatment Directive [75] which was developed after eutrophication was recognised as a water pollution problem in most European rivers [76]. However, although the WFD in 2000 introduced the evaluation of the 'ecological status' as a holistic approach that considers not only the water quality but also the structure and function of the ecosystem in response to anthropogenic pressures, no indices have been developed specifically for this purpose. Since the ecological status includes the response of the ecosystem to several types of pollution and other impacts, an extra level of caution has to be taken when applying nutrient-based indices to ecosystems that are affected by other types of contamination (e.g. heavy metals, halogenated hydrocarbons, etc.) or other anthropogenic pressures such as hydrological alteration which is, nowadays, a pressure that affects many rivers and their associated estuaries [77–79]. In the Ebro estuary, ecological status values resulting from the application of existing diatom and macroinvertebrate indices were strongly influenced by the salinity gradient, and only the trophic diatom indices (TDI and TID) showed the expected response to nutrients and included a high percentage of indicator species of that estuary. Macroinvertebrate metrics were more sensitive to the hydrological pressure than to the organic pollution pressure.

Existing diatom and macroinvertebrate indices assume that an increase of stress-tolerant species will reflect significant nutrient-related disturbance as a consequence of human activities, rather than being caused by the natural environmental fluctuations that are characteristic of many transitional waters. Although our results agree with the expected dominance of eutraphentic and α- or β-mesosaprobous diatom species in transitional waters [80, 81], their distributional patterns can also indicate a high tolerance to other environmental disturbances regardless of nutrient and/or organic matter levels [82]. Moreover, high abundances of the so-called stress-tolerant species do not always have to imply a decrease in ecological status, as stated by the 'estuarine quality paradox' [7, 83]. In the Ebro estuary, results suggest that the high abundance of these species, and the consequent low ecological status resulting from the indices' application, is mostly related to the fluctuating conditions caused by salt-wedge dynamics, which do not necessarily constitute altered conditions.

5 Conclusions

Nowadays, the main anthropogenic pressure in the Ebro estuary is the alteration of the hydrological regime mainly caused since the 1960s by reservoir functioning and increased agricultural activities, which result in stable low flows and increased salt-wedge presence. During the last two decades, there has been a trend of decreasing eutrophication and pollution but an increase in hydrological alteration, which poses additional difficulties in developing bioindicators for the assessment of river-dominated estuaries.

Our results show that although benthic diatoms and macroinvertebrates have potential as bioindicators of altered hydrological conditions in the Ebro estuary, the direct application of the existing indices to assess ecological status of the Ebro estuary and other salt-wedge estuaries cannot be recommended. This is mainly explained by the fact that most of the indices were originally developed to assess water quality of the North and Central Europe rivers or coastal marine waters, but none were designed to assess the ecological status of river-dominated estuaries, and therefore none of the tested indices consider the main ecological conditions characteristic of these ecosystems. Neither do they take into account other anthropogenic pressures besides eutrophication, such as flow regulation and other pollution sources. In addition, in salt-wedge estuaries, it is difficult to discern natural from anthropogenic stressors, because the increase in environmental stability leads to higher complexity in biological communities and thus some bioindicators may show scores indicating better ecological status under impacted conditions than under natural conditions, which is an expression of a phenomenon known as 'estuarine quality paradox'.

This study provides the basis for overcoming the difficulties of properly assessing the ecological status of river-dominated estuaries that are undergoing hydrological alteration, but more research is needed to develop specific bioindicators, especially for the case of organic pollution. Some promising results were obtained regarding the response of some metrics to hydrological alteration, but a more detailed analysis is needed to make sure that the response of the metrics is fully due to the hydrological alteration gradient or there are other factors involved.

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New Tools to Analyse the Ecological Status of Mediterranean Wetlands and Shallow Lakes

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Abstract The efforts done in Catalonia (Spain) to assess the ecological status of Mediterranean wetlands and shallow lakes are described. The term wetland includes all shallow lentic waterbodies, temporary or permanent, where light reaches the bottom allowing the development of primary producers at the maximum water depth. Two water quality indexes and one habitat condition rapid assessment were developed. The first quality index ($QAELS^{e}_{2010}$) is based on the sensitivity of microcrustaceans (cladocerans, copepods and ostracods) and the richness of crustaceans and insects found in these habitats; the second one (*EQAT*) uses the composition of Chironomidae pupal exuviae. Rapid assessment of habitat condition (*ECELS* index) considers wetland hydromorphological aspects, the presence of human pressures in the surroundings and the conservation status of the wetland vegetation. Some data of the current ecological status of Mediterranean wetlands in Catalonia are also provided.

Keywords Chironomidae, Crustaceans, *ECELS*, *EQAT*, Habitat condition, Insects, *QAELS*, Shallow lakes, Transitional waters, Wetlands - WFD

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Contents

1 Introduction

Since the implementation of the Water Framework Directive in 2000 (WFD, Directive 2000/60/EC), several efforts have been done in the description of parameters related to the ecological status of shallow lakes and wetlands and the design of efficient tools based on biological elements for its assessment (e.g. [1–7]). During this process, some difficulties arose in the development of criteria and methodological standards on good environmental status of shallow waters. Sediment proximity makes nutrient concentrations often more dependent on water – sediment equilibria than on nutrient inputs [8], making difficult to distinguish between anthropogenic eutrophication and natural eutrophication [9]. In Mediterranean wetlands, water level fluctuations and the lack of water inputs during most of the year cause an endorheic process of nutrient accumulation [10, 11], accentuated in temporary habitats during desiccation. Moreover, in Mediterranean transitional waters (i.e. estuaries, lagoons and coastal wetlands), the low tidal influence favours water confinement, making nutrient contents and nutrient balances more dependent on internal loading than on external water inputs [12–14].

Following the guidelines of the WFD, several indices have been developed using aquatic invertebrate fauna as indicators to assess the ecological status of Mediterranean shallow lentic ecosystems. Some of them use the sensitivity of species composition (e.g. [15]) or are based on higher taxonomic levels (e.g. [16–19]). Other approaches use alternatives to invertebrate species composition, such as body size [20, 21] or percentages of some functional groups [22]. Within aquatic invertebrates, several properties make crustaceans and insects suitable for their use in the ecological status assessment of wetlands and shallow lakes [23]: they appear in all lentic environments in fresh and transitional waters and are easy to capture; their assemblages vary with differences in trophic state; they respond rapidly to disturbance; and the relationships between their assemblages and both phytoplankton and macrophytes are well documented [24–30].

In this chapter, we summarise the efforts done in Catalonia (Spain) to assess the ecological status of Mediterranean wetlands and shallow lakes. We describe two water quality indexes: the first one ($QAELS^{e}_{2010}$) is an improvement of a water quality index already published [23], based on the sensitivity of microcrustaceans (cladocerans, copepods and ostracods) and the richness of crustaceans and insects; the second one (*EQAT*) is a proposal based on the composition and sensitivity of Chironomidae assemblages through the use of pupal exuviae described in Cañedo-Argüelles et al. [31]. We also include a rapid assessment method to determine the habitat condition of wetlands, developed by Sala et al. [32].

2 Typologies and Reference Conditions

The spatial approach is the underlying methodological principle of the WFD for the development of biotic indices to assess the ecological status of surface waters. The concept is that waterbodies can be classified into units with homogenous characteristics, thus belonging to a similar functional type with comparable biological communities. The principle behind this approach is that the less the functional and biotic heterogeneity within identified types, the higher the accuracy of the employed biological indicators. The WFD offers two options to classify waterbodies of surface waters, both of them use only abiotic descriptors to define typologies. The resulting classification of surface waterbodies is based on the assumption that an abiotic typology is adequate to stratify biological communities. However, there are few examples of efforts to validate this assumption in wetlands [23, 33]. Moreover, several proposals exist for Mediterranean lentic and shallow waters using abiotic variables, chlorophyll-*a* abundance or vegetation composition (e.g. [34–37]).

For the identification of types in Catalan wetlands, we follow Boix et al. [23]. This classification splits wetlands according to salinity and water permanence, and its effectivity to identify different invertebrate communities has been validated [38]. Salinity discriminates between meso-hyperhaline waters (conductivity $> 5\ mS \cdot cm^{-1}$) and fresh oligohaline waters (conductivity $< 5\ mS \cdot cm^{-1}$). Meso-hyperhaline wetlands are different if salinity comes from marine origin (thalassohaline wetlands) or from endorheic concentration of salts in arid or semiarid regions (athalassohaline wetlands). Regarding fresh oligohaline wetlands, permanent and temporary waterbodies contain different invertebrate fauna. Thus, four wetland types were discriminated: 1-thalassohaline (TA), 2-athalassohaline, 3-freshwater permanent (PF) and

4-freshwater temporary (TF). Athalassohaline wetlands are very scarce in Catalonia and are not considered further.

For each waterbody type, the basic functional unit, reference conditions are formulated and the deviation from these conditions provides the measure of the ecological status. The reference conditions can be defined in different ways [39]. If reference conditions are not available (the most common situation in the case of wetlands), one option is to use best available least-disturbed conditions resulting in unequal thresholds for less and more impacted biological assemblage types. However, the WFD requires standardised reference conditions showing no, or only minor, anthropogenic alterations. Another way of defining reference conditions is the availability of historical data when anthropogenic impacts were nonexistent or very low. In both cases, present and historical data to define reference conditions and information on pressures is necessary to distinguish between reference and impacted sites and for calibrating or scoring of metrics. This information should be expressed by different variables that should quantify the environmental quality of the surface waterbodies taking into account different types of pressures and impacts (water pollution, hydromorphological quality, etc.). It is, thus, easy to understand that classifying waterbodies and defining reference conditions should be two independent procedures; otherwise, the response of biological indicators to pressures and impacts will not be accurate. Therefore, pressure or impact variables should not be used to define typologies (i.e. the waterbodies' typology should be done with undisturbed waterbodies' datasets and using exclusively variables that cannot be modified by anthropogenic activities). Only then, proper reference conditions can be formulated for each waterbody type.

Wetlands have been lost and disturbed more rapidly than other ecosystems, and much of the global wetland area that remains is degraded (Millennium Ecosystem Assessment [40, 41]). Worldwide, an estimated half of the total wetland area has been lost due to anthropogenic activities [41]. Moreover, the historical information on wetlands is very scarce and often nonexistent, especially in Mediterranean areas. Thus, developing a wetland typology is a challenging task. One of the most reasonable ways to cope with these difficulties is to use expert judgement to define and evaluate the relevant abiotic variables, which, as previously said, should not be the same used to evaluate anthropogenic impacts and pressures. This approach supposes a deep knowledge of the ecological functioning of the wetlands to be assessed. Other more objective approaches analyse the influence of wetland environmental variables on the spatiotemporal patterns of their fauna [37, 38, 42, 43].

3 Water Quality Assessment Using Crustaceans and Insects: The $QAELS^e_{2010}$ Index

3.1 Background

Boix et al. [23] developed the *QAELS* water quality index for wetlands and shallow lakes carried out in Catalonia, based on microcrustacean sensitivity complemented with richness of crustaceans and insects. Later some improvements were done in the quality coefficients of the different species and in the definition of the quality category thresholds. Here we describe the resulting $QAELS^e_{2010}$ index.

3.2 Sampling Procedure

For the construction of the water quality $QAELS^e_{2010}$ index, we used data of 200 Mediterranean wetlands located throughout Catalonia (Fig. 1). This includes wetlands, shallow lakes, lagoons, ponds and pools, that is, all lentic waterbodies, temporary or permanent, that are shallow enough that light reaches the bottom allowing the presence of macrophytes or other primary producers at the maximum water depth [33, 44]. From here on, we will use the term "wetlands" to refer these shallow water ecosystems. Wetlands were sampled once (132 waterbodies) in late spring or twice a year (68 waterbodies) in late winter and late spring. All wetlands sampled were below 800 m a.s.l. to ensure they were under Mediterranean climatic conditions. Therefore, those located in mountain and alpine climatic areas, above

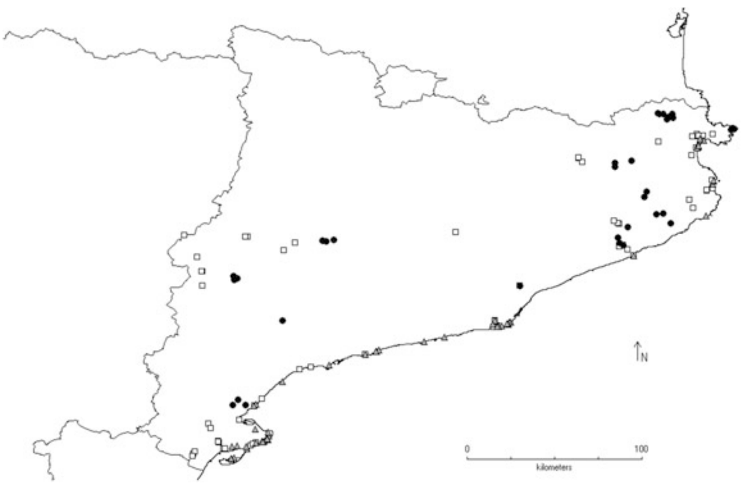

Fig. 1 Location of the studied wetlands (*triangles*, thalassohaline wetlands; *white squares*, permanent freshwater wetlands; *black points*, temporary freshwater wetlands)

800 m a.s.l., were not considered. Wetlands were previously classified by types following Boix et al. [23]. Thus 76 waterbodies were thalassohaline, 79 were freshwater permanent and 45 were freshwater temporary. Temperature, conductivity, percentage of oxygen saturation and pH were measured in situ. Chlorophyll-*a* was extracted using 80% methanol, after filtering water samples (Whatman GF/C filters), and measured following Talling and Driver [45]. Analyses of dissolved inorganic nutrients (ammonium, nitrite, nitrate and phosphate) were carried out from filtered samples and total nutrients (total nitrogen and total phosphorus) from unfiltered samples, following Grasshoff et al. [46].

Invertebrate sampling was performed as described in Boix et al. and ACA [23, 47], using a 20 cm diameter dip-net (mesh size 250 μm). At each wetland, three sweeps of dip-net "pushes" per visit were carried out along transects. Each sweep consisted of 20 dip-net "pushes" in rapid sequence, to cover all the different habitats in the littoral zone of the wetland. Only the organisms from the first sweep were used to estimate the relative abundances of microcrustaceans, whereas all sweeps were used to calculate the taxon richness. Samples were preserved in 10% formalin. All crustaceans and insects were identified to species level, or to the lowest taxonomic level possible, except for dipterans, which were always identified to family level.

3.3 *Building* $\mathrm{QAELS}^{e}_{2010}$ *Index*

The $QAELS^{e}_{2010}$ index consists of two components: the first one is obtained from the composition of microcrustaceans and the sensitivity of their different species to water quality ($ACCO_{2010}$ value); the second one is related to crustacean and insect richness (*RIC* value). Microcrustaceans and macroinvertebrates strongly differ in abundance, and a correct estimation of the abundance of both faunal groups may be highly time-consuming. Thus the $ACCO_{2010}$ value only considers microcrustacean taxa, because a rapid estimation of abundance is preferred in bio-assessment indices. However, when estimating richness, it is better to include as many faunal groups as possible [23], since a large number of taxa offer a spectrum of responses to environmental stresses [48]. That's why the *RIC* value includes crustacean and insect richness.

Microcrustacean sensitivities to build the $ACCO_{2010}$ value were obtained by means of a partial canonical correspondence analysis (PCCA). A different PCCA analysis was carried out for each wetland type. In the microcrustacean matrix, the relative abundance of each species was square-root transformed and rare species were downweighted in order to reduce their influence in the analysis. The water quality variables matrix used in PCCA was composed by a unique variable, the *TRIX* index, described by Vollenweider et al. [49]:

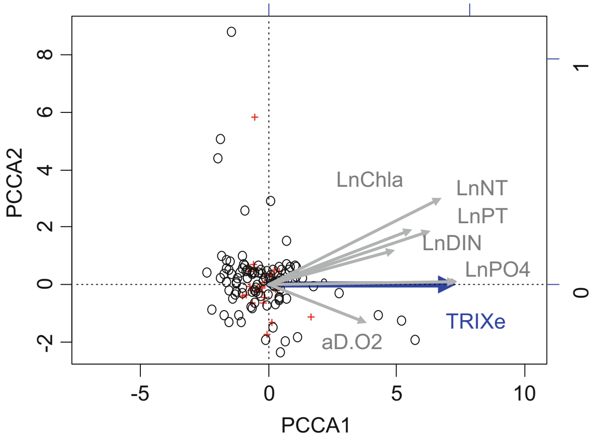

Fig. 2 Results of the PCCA analysis using the *TRIX* index (*blue arrow*) as variable indicative of water quality in permanent freshwater wetlands. Other variables related to water quality are not considered in the analysis, but included in the plot (*grey arrows*) as supplementary variables (*Chla* chlorophyll-*a*, *aD.O2* absolute deviation of 100% of oxygen saturation (see text), *DIN* dissolved inorganic nitrogen, *NT* total nitrogen, *PO4* soluble reactive phosphate, *PT* total phosphorus). *Circles* and *crosses* represent samples and species position, respectively. Similar plots were built for other wetland types (thalassohaline and temporary freshwater wetlands)

$$TRIX = \frac{[\log_{10}(\text{Chla} \cdot \text{aD.O2} \cdot \text{DIN} \cdot \text{Pt}) + 1.5]}{1.2}, \tag{1}$$

where Chla, DIN and Pt are the chlorophyll-*a*, the dissolved inorganic nitrogen and the total phosphorus concentrations ($\text{mg} \cdot \text{L}^{-1}$) and aD.O2 is the absolute deviation of the percentage of oxygen saturation (i.e. the absolute value of 100% O_2 saturation). This index has been widely used in water quality assessment, especially in transitional waters [50–52]. Variability caused by variables not necessarily related to water quality, such as temperature or conductivity, was removed from the PCCA analysis by entering them as covariables.

The first PCCA axis was strongly related to *TRIX* index in each of the three different wetland types. Other environmental variables related to water quality were included as supplementary variables, such as chlorophyll-*a*, total and dissolved nitrogen and phosphorus, related to the same first PCCA axis (Fig. 2). Thus, we used the microcrustacean species scores in this first PCCA axis as a measure of species sensitivity. Only species with occurrences >1% were considered indicator species. Microcrustacean indicator species were sorted by their scores in the first PCCA axis. Scores were distributed in ten categories, and a value between 1 and 10 was assigned to each indicator species. This rescaled score is the "quality coefficient" used for the computation of the $ACCO_{2010}$ value. Extreme and anomalous scores for the interval (values >1.5 times the interquartile range) were not taken into account for the creation of the ten categories. Quality coefficients for a given species differ among wetland types, and some taxa may be indicator in some types and not in others. The final $ACCO_{2010}$ value is obtained by means of the following equation:

$$ACCO = \sum_{i=1}^{j} k_i \cdot n_i; \quad n_i = \frac{N_i}{N_{\text{tot}}}, \tag{2}$$

where

$i =$ each taxon with a weight in the analysis >1% (indicator species)
$j =$ number of taxa with a weight in the analysis >1%
$n_i =$ relative abundance of the species i
$k_i =$ quality coefficient of the species i
$N_i =$ abundance of the species i
$N_{\text{tot}} =$ sum of the abundance of the species with a weight in the analysis >1%

To determine species quality coefficients and their robustness, for each microcrustacean species, we did 100 additional iterations of the same PCCA analyses (one per each wetland type) but randomly deleting 5% of the samples used. Quality coefficients k_i were then obtained by the weighted average of the quality coefficients of these 100 PCCA analyses, rescaled to a 0–10 value and rounded to the nearest integer. Figure 3 shows the results of the variability in coefficient estimation using this procedure. Results indicate a high robustness of quality coefficients in those species that show a narrow range of quality coefficients variability (see *Megacyclops viridis* or *Cypria ophtalmica* in Fig. 3) and a lower robustness in those species with wider quality coefficient variability (see *Simocephalus exspinosus* or *Eucyclops serrulatus* in Fig. 3).

The *RIC* value is used as a non-biased estimation of crustacean (micro- and macrocrustaceans) and insect richness (presence–absence data). *RIC* is calculated as the sum of the number of crustacean genera, the number of families of immature stages of insects (nymphs, pupae and larvae) and the number of genera of adult Coleoptera and Heteroptera. The resulting $QAELS_{2010}$ index is the combination of $ACCO_{2010}$ and *RIC* values, which differ depending on wetland types:

$$\text{Thalassohaline wetlands}: \quad QAELS_{2010} = (1 + ACCO_{2010}), \tag{3}$$

$$\text{Permanent freshwater wetlands}: \quad QAELS_{2010} = (1 + ACCO_{2010}) + \log_{10}(RIC + 1), \tag{4}$$

$$\text{Temporary freshwater wetlands}: \quad QAELS_{2010} = (1 + ACCO_{2010}) + \log_{10}(RIC + 1). \tag{5}$$

RIC is not used for $QAELS_{2010}$ computation in thalassohaline wetlands because *RIC* inclusion reduces correlation between $QAELS_{2010}$ and the variables related to water quality (Table 1). In thalassohaline ecosystems freshwater inputs also imply nutrient inputs and can be considered as disturbances that affect community structure

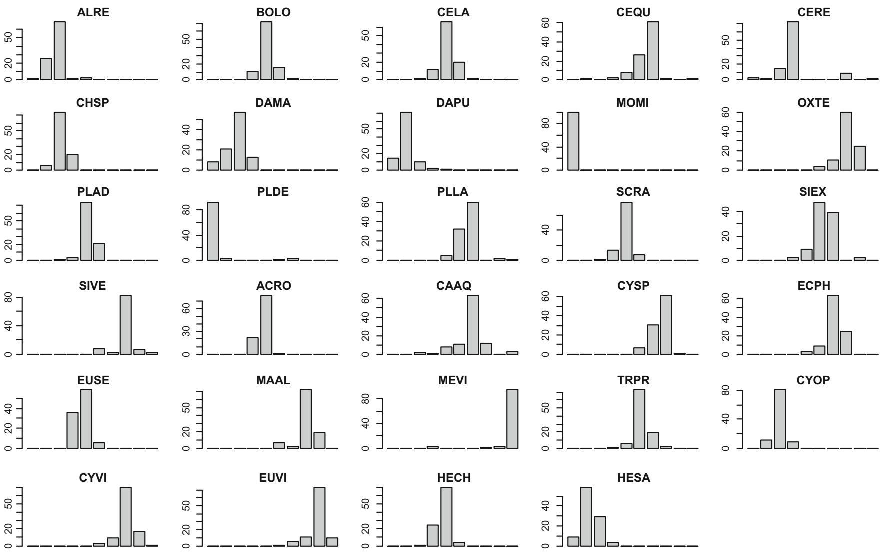

Fig. 3 Variation of quality coefficients (k_i in Eq. 1) in permanent freshwater wetlands after 100 iterations of the PCCA analysis, where randomly 5% of samples used was deleted. *Columns* represent the standardised value of k_i, from 1 (*left column*) to 10 (*right column*). *Column height* indicates the number of PCCAs where the species achieved a determinate k_i score. Species codes: CLADOCERANS—ALRE, *Coronatella rectangula*; BOLO, *Bosmina longirostris*; CELA, *Ceriodaphnia laticaudata*; CEQU, *C. quadrangula*; CERE, *C. reticulata*; CHSP, *Chydorus sphaericus*; DAMA, *Daphnia magna*; DAPU, *D. pulicaria*; MOMI, *Moina micrura*; OXTE, *Oxyurella tenuicaudis*; PLAD, *Pleuroxus aduncus*; PLDE, *P. denticulatus*; PLLA, *P. laevis*; SCRA, *Scapholeberis rammneri*; SIEX, *Simocephalus exspinosus*; SIVE, *S. vetulus*. COPEPODS—ACRO, *Acanthocyclops* gr. *robustus-vernalis*; CAAQ, *Calanipeda aquaedulcis*; CYSP, *Cyclops* sp.; ECPH, *Ectocyclops phaleratus*; EUSE, *Eucyclops serrulatus*; MAAL, *Macrocyclops albidus*; MEVI, *Megacyclops viridis*; TRPR, *Tropocyclops prasinus*. OSTRACODS—CYOP, *Cypria ophtalmica*; CYVI, *Cypridopsis vidua*; EUVI, *Eucypris virens*; HECH, *Herpetocypris chevreuxi*; HESA, *Heterocypris salina*

[53–55]. Freshwater inputs usually increase the number of species in those thalassohaline waters [43, 56]. Thus, an increase in species richness in these ecosystems may indicate a higher degree of disturbance related to higher nutrient concentrations coming with freshwater inputs.

Because maximum values of $QAELS_{2010}$ index differ in the different wetland types, each $QAELS_{2010}$ index was standardised with the division by the maximum $QAELS_{2010}$ value reached for a specific wetland type:

$$\text{Thalassohaline wetlands:} \quad QAELS^{e}_{2010} = \frac{QAELS_{2010}}{10.97}, \qquad (6)$$

Table 1 Spearman correlation coefficients between variables related to trophic state and the *ACCO* or the *ACCO* + *RIC* indexes in permanent freshwater (PF), temporary freshwater (TF) and thalassohaline (TA) wetlands

		TN	TP	SRP	DIN	Chla	*TRIX*
PF	*ACCO*	n.s.	−0.27***	−0.20*	n.s.	−0.32***	−0.38***
	ACCO + *RIC*	n.s.	−0.35***	−0.31***	n.s.	−0.34***	−0.36***
TF	*ACCO*	−0.42**	−0.27*	−0.26*	−0.42**	−0.28*	−0.57***
	ACCO + *RIC*	−0.40*	n.s.	−0.32*	−0.39*	−0.34*	−0.57***
TA	*ACCO*	−0.48***	n.s.	−0.43***	n.s.	−0.22*	−0.42***
	ACCO + *RIC*	−0.49***	n.s.	−0.41***	n.s.	−0.24*	−0.43***

Note that the addition of the *RIC* value does not increase the correlation in TA wetlands. All trophic variables, except the *TRIX* index, were log transformed

TN total nitrogen, TP total phosphorus, SRP soluble reactive phosphate, DIN dissolved inorganic nitrogen, Chla chlorophyll-*a*

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; *n.s.* not significant ($p > 0.05$)

$$\text{Permanent freshwater wetlands} : QAELS^{e}_{2010} = \frac{QAELS_{2010}}{12.44}, \tag{7}$$

$$\text{Temporary freshwater wetlands} : QAELS^{e}_{2010} = \frac{QAELS_{2010}}{11.08}, \tag{8}$$

where the divisor number corresponds to the maximum $QAELS_{2010}$ value obtained in each wetland type.

3.4 Required Taxonomic Resolution

Boix et al. [23], in their previous version of the *QAELS* index ($QAELS_{2004}$), showed that low levels of resolution in microcrustacean taxa determination were not acceptable, since correlations between the index obtained at species level and the index computed using taxonomic determination at main group or at family level gave low correlations (even not significant in some cases). When using the resolution at genus level, correlation values oscillated between 0.667 and 0.986, depending on wetland types. According to this, we correlated the $ACCO_{2010}$ values using taxonomic resolutions at species and genus level (Fig. 4) and found a high correlation for thalassohaline and permanent freshwater wetlands, but a low one for temporary freshwater wetlands. Thus, results suggest that a resolution at genus level is suitable for thalassohaline and permanent freshwater wetlands, but for temporary freshwater wetlands, the highest level of resolution is needed. Thus, in order to simplify the computation of $ACCO_{2010}$ index, we propose a taxonomic resolution at genera level for thalassohaline and permanent freshwater waterbodies, but at

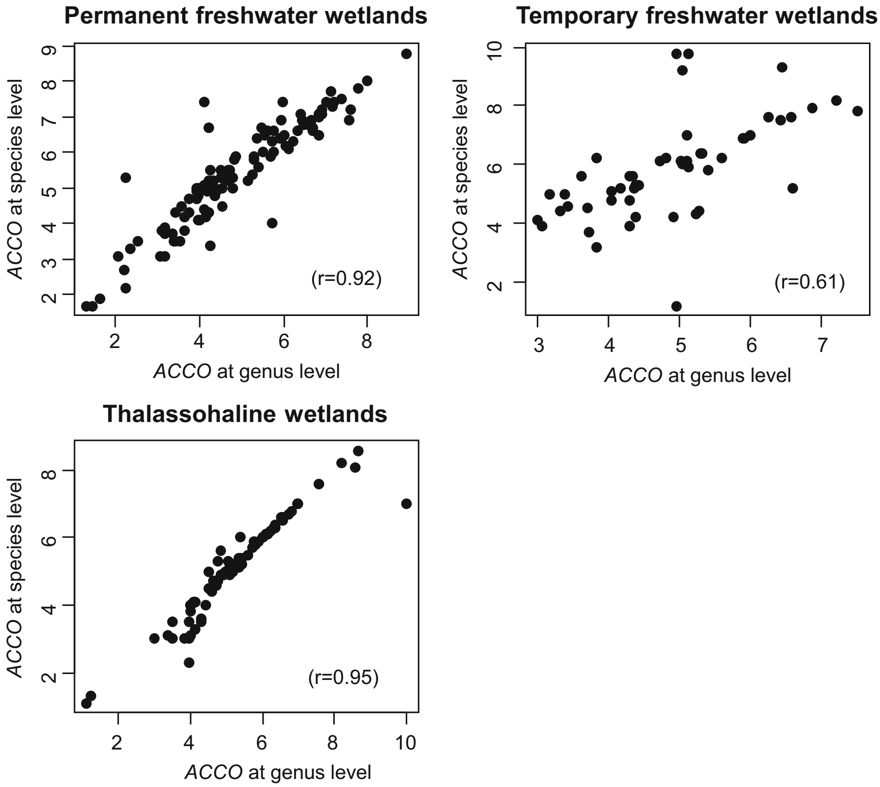

Fig. 4 Correlations between the *ACCO* indexes estimated with different taxonomic resolutions

species level for temporary freshwaters. Quality coefficients (k_i) obtained for each microcrustacean taxa are listed in Tables 2 and 3.

3.5 *Water Quality Thresholds*

To define the $QAELS^e_{2010}$ boundaries that separate the five categories proposed by the WFD (high, good, moderate, poor or bad), we follow the recommendations of the REFCOND document [57]. Five different Wallin et al. [57] proposals were tested, listed in Table 4. To select which of the five methods was the most suitable, we performed Spearman correlations between the water quality classes and variables dealing with trophic state (nutrients, chlorophyll-*a*, *TRIX* value). Results obtained in the five different proposals gave significant relationships between the water quality classes and the trophic-related variables. We chose proposal 5, which gave the highest correlation values (Fig. 5). The resulting category boundaries for each wetland type are listed in Table 5.

Table 2 Quality coefficients (k_i in Eq. 1) of each indicator genus for the computation of the $ACCO_{2010}$ value in each wetland type

	TA	PF
CLADOCERA		
Alona	–	8
Bosmina	–	5
Ceriodaphnia	–	4
Chydorus	5	3
Coronatella	–	8
Daphnia	1	2
Moina	–	1
Oxyurella	–	8
Pleuroxus	3	5
Scapholeberis	–	8
Simocephalus	4	7
COPEPODA		
Acanthocyclops	4	4
Calanipeda	6	6
Canuella	4	–
Cletocamptus	4	–
Cyclops	7	8
Diacyclops	7	–
Ectocyclops	–	7
Eucyclops	3	4
Eurytemora	7	–
Halicyclops	5	–
Harpacticus	7	–
Macrocyclops	–	8
Megacyclops	–	10
Mesochra	10	–
Nitokra	7	–
Paracyclops	–	1
Pseudonychocamptus	5	–
Tisbe	3	–
Tropocyclops	9	6
OSTRACODA		
Cypria	–	3
Cyprideis	5	–
Cypridopsis	7	8
Eucypris	6	8
Herpetocypris	–	4
Heterocypris	4	1
Loxoconcha	5	–
Sarscypridopsis	1	–
Xestoleberis	6	–

(–) Genera with no indicator value in this wetland type
TA thalassohaline wetlands, *PF* permanent freshwater wetlands

Table 3 Quality coefficients (k_i in Eq. 1) of each indicator species for the computation of the $ACCO_{2010}$ value in temporary freshwater wetlands, where a taxonomical resolution to species level is required

CLADOCERA	
Coronatella rectangula	3
Ceriodaphnia quadrangula	5
Ceriodaphnia reticulata	3
Chydorus sphaericus	6
Daphnia curvirostris	10
Daphnia magna	3
Daphnia obtusa	1
Daphnia pulicaria	7
Moina brachiata	5
Simocephalus exspinosus	6
Simocephalus vetulus	7
COPEPODA	
Acanthocyclops gr. *robustus-vernalis*	5
Canthocamptus staphylinus	9
Cyclops sp.	5
Diacyclops bicuspidatus	8
Diacyclops bisetosus	4
Diaptomus cyaneus	10
Megacyclops viridis	5
Metacyclops minutus	7
Mixodiaptomus incrassatus	7
Mixodiaptomus kupelwieseri	6
Neolovenula alluaudi	4
OSTRACODA	
Cyclocypris ovum	4
Cypridopsis vidua	8
Eucypris virens	5
Herpetocypris chevreuxi	7
Heterocypris barbara	4
Heterocypris incongruens	5
Plesiocypridopsis newtoni	4

4 The Use of Chironomidae as a Bioindicator: The *EQAT* Index

4.1 Background

Within the aquatic insects' assemblages found in the Mediterranean lagoons and wetlands of Spain, Chironomidae tend to be the most abundant and rich in species [58–61]. Chironomidae larvae are present in all habitats and have a great variety of biological traits; for example, *Chironomus* burrows in the sediment collecting organic matter that is being accumulated as fine sediment, while *Psectrocladius* tends to live attached to the helophytes and the submerged vegetation, feeding on

Table 4 Proposals for category boundaries tested

Proposal 1	All the locations of a given type were considered together
	High: $QAELS^{e}_{2010} > \mathrm{P90}$
	Good: $\mathrm{P75} < QAELS^{e}_{2010} < \mathrm{P90}$
	Moderate: $\mathrm{P50} < QAELS^{e}_{2010} < \mathrm{P75}$
	Poor: $\mathrm{P25} < QAELS^{e}_{2010} < \mathrm{P50}$
	Bad: $QAELS^{e}_{2010} < \mathrm{P25}$
Proposal 2	Only locations under reference conditions (ref) were considered, from which the standard deviation (SD) is calculated
	High: $QAELS^{e}_{2010} > \mathrm{P90ref}$
	Good: $\mathrm{P90ref} - \mathrm{SD} < QAELS^{e}_{2010} < \mathrm{P90ref}$
	Moderate: $\mathrm{P90ref} - 2 \cdot \mathrm{SD} < QAELS^{e}_{2010} < \mathrm{P90ref} - \mathrm{SD}$
	Poor: $\mathrm{P90ref} - 3 \cdot \mathrm{SD} < QAELS^{e}_{2010} < \mathrm{P90ref} - 2 \cdot \mathrm{SD}$
	Bad: $QAELS^{e}_{2010} < \mathrm{P90ref} - 3 \cdot \mathrm{SD}$
Proposal 3	Only locations under reference conditions (ref) were considered, from which the standard deviation (SD) is calculated
	High: $QAELS^{e}_{2010} > \mathrm{P90ref}$
	Good: $\mathrm{P90ref} - \mathrm{SD} < QAELS^{e}_{2010} < \mathrm{P90ref}$
	Moderate to bad categories, obtained by dividing the remaining values of the index in equal parts
Proposal 4	Only locations under reference conditions (ref) were considered, from which the standard deviation (SD) is calculated
	High: $QAELS^{e}_{2010} > \mathrm{P90ref}$
	Good: $\mathrm{P90ref} - \mathrm{SD} < QAELS^{e}_{2010} < \mathrm{P90ref}$
	Percentiles of no reference locations (no_ref) were used for the remaining categories.
	Moderate: $\mathrm{P50no\_ref} < QAELS^{e}_{2010} < \mathrm{P90ref} - \mathrm{SD}$
	Poor: $\mathrm{P25no\_ref} < QAELS^{e}_{2010} < \mathrm{P50no\_ref}$
	Bad: $QAELS^{e}_{2010} < \mathrm{P25no\_ref}$
Proposal 5	Only locations under reference conditions (ref) were considered for the high category. Percentiles of no reference locations (no_ref) were used for the remaining categories
	High: $QAELS^{e}_{2010} > \mathrm{P90ref}$
	Good: $\mathrm{P75no\_ref} < QAELS^{e}_{2010} < \mathrm{P90ref}$
	Moderate: $\mathrm{P50no\_ref} < QAELS^{e}_{2010} < \mathrm{P75no\_ref}$
	Poor: $\mathrm{P25no\_ref} < QAELS^{e}_{2010} < \mathrm{P50no\_ref}$
	Bad: $QAELS^{e}_{2010} < \mathrm{P25no\_ref}$

P percentiles, *SD* standard deviation

fresh algae [62]. Moreover, they are present over wide environmental ranges (including salinity), with some species being very sensitive to pollution, whereas others can survive in anoxic and polluted environments. Therefore, they have been successfully used as indicators of water quality in rivers [63–65] and lakes [66–68].

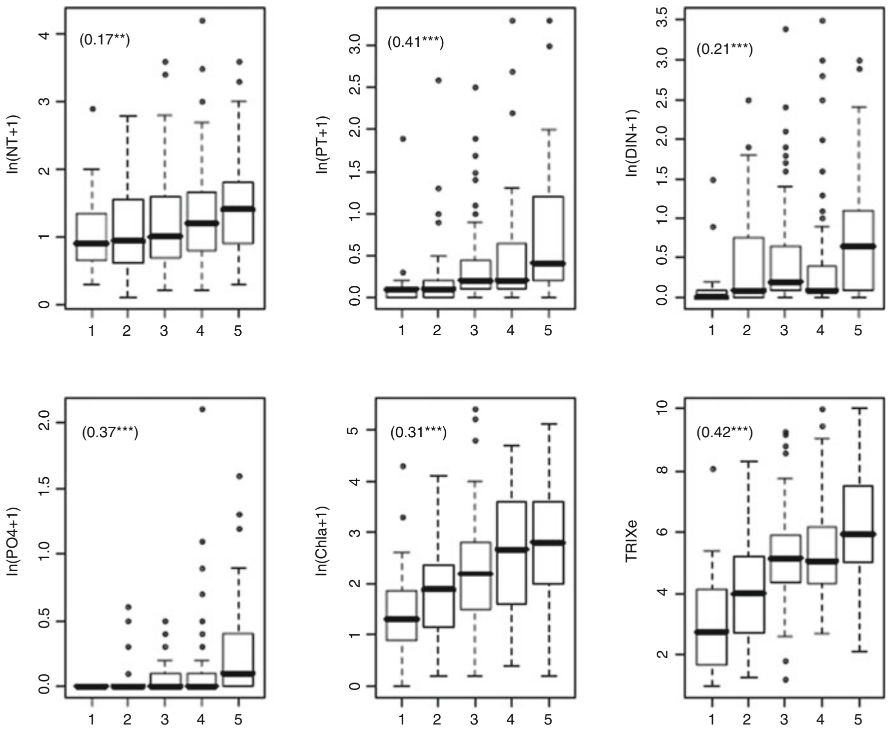

Fig. 5 *Boxplots* showing the variability of the different environmental variables related to trophic state for each $QAELS^{e}_{2010}$ quality class (*1*, high; *2*, good; *3*, moderate; *4*, poor; *5*, bad). $QAELS^{e}_{2010}$ quality classes were obtained as described in Table 4 proposal 5. The overall Spearman correlation coefficient is also included (**, $p < 0.01$; ***, $p < 0.001$). Codes of environmental variables as in Fig. 2

The *EQAT* biomonitoring tool is based on Chironomidae and has the advantage that it is cost-effective (cheap, involving low time consumption in the field and the laboratory and easy to use) and that it integrates all the habitats within the ecosystem. The tool can be easily used by trained technicians to assess water quality status on a regular basis and to help identify those waterbodies being at risk of failing to meet their environmental objectives according to the WFD.

4.2 How to Use EQAT*?*

The Chironomidae (Diptera) are holometabolous insects with four life stages (egg, larva, pupa and adult). Larvae grow in the water associated to the different available habitats. Eventually the late fourth instar larvae develop wing pads, moult to pupae and then swim to the water surface where adults cast their pupal skin (exuviae) and emerge to mate [69]. Since all the chironomid larvae inhabiting a given waterbody

Table 5 Boundaries of the different $QAELS^{e}_{2010}$ quality classes in permanent freshwater (PF), temporary freshwater (TF) and thalassohaline (TA) wetlands

Quality class	PF	TF	TA
High	$QAELS^{e}_{2010} \geq 0.86$	$QAELS^{e}_{2010} \geq 0.89$	$QAELS^{e}_{2010} \geq 0.72$
Good	$0.58 \leq QAELS^{e}_{2010} < 0.86$	$0.68 \leq QAELS^{e}_{2010} < 0.89$	$0.62 \leq QAELS^{e}_{2010} < 0.72$
Moderate	$0.51 \leq QAELS^{e}_{2010} < 0.58$	$0.56 \leq QAELS^{e}_{2010} < 0.68$	$0.55 \leq QAELS^{e}_{2010} < 0.62$
Poor	$0.39 \leq QAELS^{e}_{2010} < 0.51$	$0.45 \leq QAELS^{e}_{2010} < 0.56$	$0.46 \leq QAELS^{e}_{2010} < 0.55$
Bad	$QAELS^{e}_{2010} < 0.39$	$QAELS^{e}_{2010} < 0.45$	$QAELS^{e}_{2010} < 0.46$

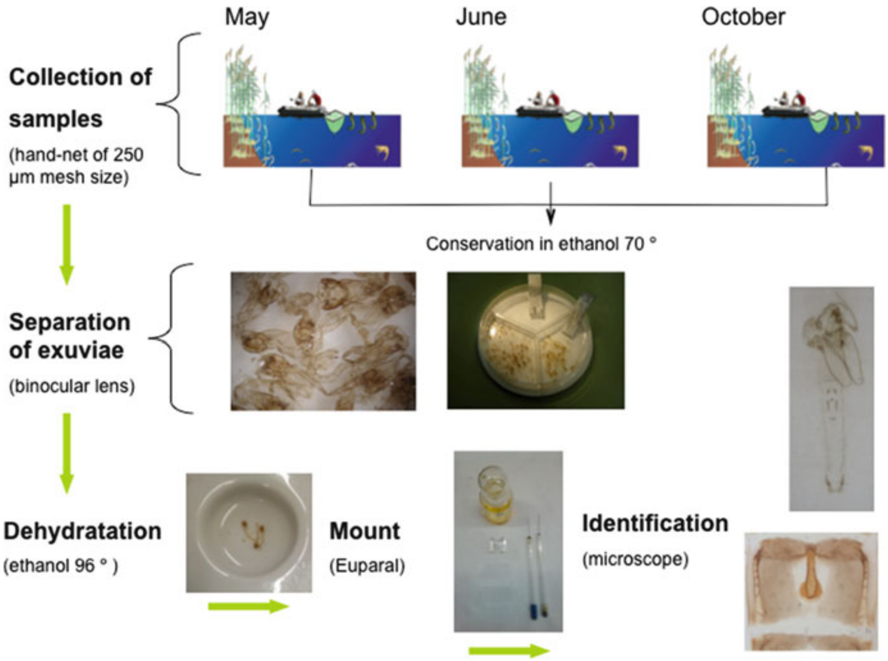

Fig. 6 Methodological scheme for the collection and processing of Chironomidae exuviae needed to apply the *EQAT* index. The first step is the collection of samples (ideally performed three times a year, coinciding with the periods of maximum emergence of Chironomidae), and *green arrows* indicate each next methodological step

will eventually undergo this metamorphosis, pupal exuviae collection has a great potential for characterising the whole chironomid community. Collection of samples is easy and fast. First the areas of accumulation of organic matter (characterised sometimes by the presence of white foam) must be identified, and then chironomid exuviae can be collected there by sweeping a 250 μm mesh size hand net along the shore. Ideally the samples should be collected on three different occasions (May, June and October), which are likely to comprise the maximum emergence periods of chironomids in Mediterranean lagoons [70]. The three samples can be merged and considered as one. Once collected, the samples must be preserved in ethanol 70° and taken to the laboratory. In the laboratory the samples must be sieved through a 250 μm mesh and placed in a Petri dish. Chironomid pupal exuviae must be removed from debris and identified to family level using binocular magnifying lens. Then they must be dehydrated using ethanol 96°, mounted permanently in Euparal on a microscope slide and identified to genus [71] using a high-magnification (400×) microscope (Fig. 6). We use genus level instead of species level identification because it is considerably less time-consuming and the genus-level index is equally robust for detecting changes in the environment [31].

Each genus has a score according to its sensitivity to pollution. When assessing the status of a given wetland, the index value is a simple function of the relative abundance of each chironomid genus and its indicator score:

$$EQAT = \sum_{i=1}^{s} n_i \cdot k_i, \tag{9}$$

where S is the number of genera, n_i the relative abundance of the genus i and k_i its quality coefficient.

4.3 *How Was* EQAT *Designed?*

Chironomidae exuviae were collected in 37 permanent shallow waterbodies associated to several wetland areas in Catalonia, especially in the coastal area. Then genus scores were derived from the indicator species analysis (INDVAL), proposed by Dufrêne and Legendre [72]. The INVDAL analysis can be considered as a statistically robust alternative to the expert judgement, since it is based on the taxa abundance and frequency in a given group of sites (e.g. polluted versus non-polluted sites). The aim of the analysis was to obtain a score that reflected the indicator potential of each genus along the pollution gradient. For this purpose a 5-step procedure was followed:

1. To obtain the species scores based on their tolerance to pollution, the *TRIX* index (Eq. 1) was used as a pressure indicator gradient. All the sites were classified in one of the five trophic categories proposed by Vollenwieder [49]: high, good, moderate, poor or bad.
2. The INDVAL analysis assigned each genus to a most probable group of sites (high, good, moderate, poor or bad) according to the relative abundance and frequency of the genus in each of the groups. The indicator (IV) and the p values (resulting from INDVAL) indicate how strongly the genus is linked to each group (the higher the IV value and the lower the p-value, the stronger is the link between the species and the group).
3. A scaled indicator value (SIV) was obtained for each genus taking into account its IV and its p values and the IV and p values of the rest of the genera assigned to the same group of sites.
4. Once a score (SIV) was obtained for each genus, it was rescaled from 0 to 1. First the importance of each group was weighted by dividing the number of genera assigned to it by the INDVAL analysis by the total number of genera. Then the boundaries between the five groups of sites (each of them enclosing a variable number of associated indicator genera) were settled according to the weight of each group.
5. The *EQAT* can be finally calculated as a function of the relative abundance of each genus and its indicator score.

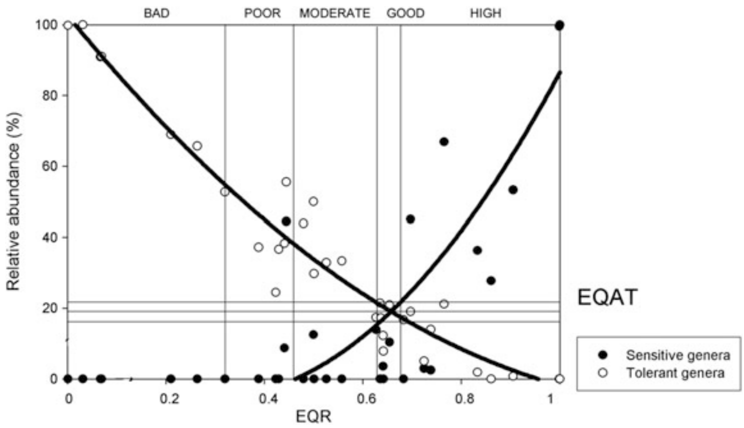

Fig. 7 Establishment of the quality class boundaries for the *EQAT* index, following Ruse [68]. On the *X*-axis the Ecological Quality Ratio (EQR), on the *Y*-axis the percentage of sensitive and tolerant genera. *Vertical lines* mark the boundaries between the quality classes: high, good, moderate, poor and bad

4.4 Establishing the Quality Class Boundaries

The final goal of the index was to assign each site to an ecological status category (high, good, moderate, poor and bad). In order to do this, the boundaries between the five categories had to be established. The class boundaries were derived from a plot of the relative frequency (%) of sensitive (genus score > 80th percentile of all the genus scores) and tolerant (genus score <20th percentile of all the genus scores) genera versus the Ecological Quality Ratio (EQR) (Fig. 7), as proposed by Ruse [68]. Since no truly reference sites could be found, the EQR was calculated taking as reference sites those that registered a maximum value of the *EQAT* index. The class boundaries were set as follows: high/good = the EQR corresponding to the relative frequency of the crossover point plus the SD; good/moderate = the EQR corresponding to the relative frequency of the crossover point minus the SD; moderate/poor = the fitted 0% of sensitive genera; poor/bad = no sensitive genera occurred and all the observed scores were well below reference (expert criteria).

4.5 Applicability

EQAT is a promising tool for monitoring the status of Mediterranean wetlands, as requested by the WFD. The index can be confidently applied in Mediterranean coastal lagoons and wetlands, but it would probably need to be adjusted in order to be used in another systems and/or geographical regions. The tool is particularly well suited for wetlands and small confined lagoons with a wide range of salinities and natural or artificial freshwater inputs, which are very common along the Mediterranean coast. These ecosystems are of great value (e.g. as a resting place for migratory birds), and at the same time, they are subjected to strong human pressures [31]. As being the last stop between the river and the sea, they receive large wastewater discharges [73] that can have great impacts on the biological communities [56]. Moreover human development tends to concentrate on the coast (e.g. Barcelona), causing problems like the hydrological alteration of the lagoons and habitat fragmentation. Therefore, the status of these coastal lagoons and wetlands needs to be continuously monitored to detect any anthropogenic impacts. In this regard, cost-effective (cheap, easy and fast) tools like *EQAT* can be very useful, since they allow the assessment of water quality of big geographic areas by nonexpert personnel within a short time period.

5 Assessing Habitat Condition: The *ECELS* Index

5.1 Background

$QAELS^{e}_{2010}$ and *EQAT* indexes reveal water quality in wetlands by means of the relationship between taxonomic composition and water nutrient charges. However, there are other aspects of wetland ecological status that are not necessarily related to water quality. This is the case of the habitat condition, which includes wetland hydromorphological aspects, human pressures or vegetation conservation status. Thus, artificial ponds built for irrigation purposes, with a very poor natural value, may have high water quality (e.g. if they are filled with pumped groundwater). On the other hand, valuable natural waterbodies may be stressed by agricultural or livestock pollution, resulting in poor water quality. Moreover, some wetlands with high water quality may be degraded in their littoral morphology or have been subjected to a strong human pressure, such as surrounding urbanisation or other human impacts. To address this question, we proposed an in situ rapid assessment method to define wetland habitat condition adapted for Mediterranean wetlands, following the rationale of other rapid assessments developed for wetlands [74] and lotic environments, such as *RCE* [75] or *QBR* [76]. This is the *ECELS* index, fully described in Sala et al. [32] and ACA [47].

5.1.1 *ECELS* Components

As described in Sala et al. [32], *ECELS* index is based on 5 components: littoral morphology, human activity, water aspect, emergent vegetation and hydrophytic vegetation. These components consider the attributes that a well-preserved wetland should have, according to a revision of widely used attributes in conservation assessments [74, 77–82], together with additional criteria that were derived from an exhaustive survey conducted by the authors.

The basin littoral morphology component (score 0–20) evaluates the slope of the wetland littoral, assuming that smooth slopes facilitate expansion of water during flooding events and allow the existence of different habitats that may increase the overall biodiversity. Anthropogenic alteration usually limits potential expansion of flooded areas with the alteration of littoral morphology and the presence of structures or activities, such as levees or burials. The human activity component (score 0–20) considers the human uses around and inside the wetland basin and in its neighbouring catchments. This includes agriculture and livestock activities, hydraulic equipment affecting water volume and turnover, transport and building facilities in the surroundings or presence of allochthonous or domestic fauna. Other aspects of human presence, such as frequency or presence of rubbish, and even those with a positive effect, such as protection and management, are considered as well. The water aspect component (score 0–10) does not try to evaluate its water quality. It only takes into account some water characteristics, such as transparency and odour, which can reflect intense anthropogenic influence. The emergent vegetation component (score 0–30) assesses the abundance and zonation of the vegetation belt, using a rough, semi-quantitative abundance approach. Species dominance, the presence of exotic plants and the presence or absence of trees around the wetland are also considered. Finally, the hydrophytic vegetation component (score 0–20) analyses the submerged and floating macrophytes using a very similar rough abundance approach. Thus, the maximum score for a wetland is 100.

By means of the analyses of these five components, *ECELS* index tries to highlight how far is the wetland from the structure, composition and zonation of a reference wetland [83–85]. A wetland with an *ECELS* score of 100 would be this with absence of human uses or structures, a gradual slope of its littoral that favours water expansion during flooding and the existence of a well-developed littoral community, a complete belt of emergent vegetation and a dense recover of submerged macrophytes. On the other hand, a wetland with an *ECELS* score of 0 might be a bad-operating aeration tank of a waste water treatment plant, with a constant surface of the flooded area, man-made control of water turnover, high turbidity, strong odour and absence of emergent and submerged vegetation. The *ECELS* scores obtained are categorised following the guidelines of the WFD as follows: high $\geq$90; good 70–89; moderate 50–69; poor 30–49; bad <30.

5.2 Applicability

ECELS index has been used elsewhere for wetland characterisation and for habitat conservation assessment [47, 86, 87]. The components of *ECELS* index are independent among themselves, each one informing about a complementary aspect. This structure makes it easy to identify the degradation problems of a particular wetland, which is useful for management purposes in order to determine the specific problems in conservation status or to identify which aspects of a managed wetland can be enhanced to reach a higher habitat condition.

Further its use in ecological status characterisation, one of the main advantages of the *ECELS* index, is that it gives a numerical value for an attribute that usually is categorical, as is the case of habitat condition. This facilitates the use of habitat condition in further numerical analyses dealing with wetland ecological functioning. In this sense, *ECELS* index has been included in environmental data matrix in the analysis of the effects of anthropogenic pressures on diatoms and macroinvertebrate species composition [88], on wetland species biodiversity patterns [89] and on dispersal ability patterns of passive dispersers in aquatic invertebrate assemblages [90].

6 Evaluating Ecological Status in Mediterranean Wetlands of Catalonia

The evaluation of the ecological status in a wetland can be obtained by means of a double entry table, combining a water quality index and a habitat condition index. Table 6 summarises the ecological status evaluation using $QAELS^{e}_{2010}$ and *ECELS* indexes.

According to estimation of ecological status in Table 6, the percentage of sampled wetlands in Catalonia that achieved the standards of high or good ecological status required by the WFD was low in permanent freshwater (14%) and thalassohaline (18.4%) wetlands (Table 7). This percentage is higher in temporary freshwater wetlands (30.8%). In the case of permanent freshwater wetlands, we did not find any wetland with high ecological status, while no temporary freshwater wetlands fall into the bad class. Having a look to the $QAELS^{e}_{2010}$ and *ECELS* percentages, we can assume that high ecological status in thalassohaline and temporary freshwater wetlands was mainly not achieved due to a low water quality (lower $QAELS^{e}_{2010}$ values), a low habitat condition being the main cause of the impairment to achieve good ecological status in permanent freshwater wetlands. These differences may be the consequence of the different human pressures that these ecosystems have suffered. Historically, humans have reduced the extension of

Table 6 Estimation of the ecological status of a wetland by means of the combination of the $QAELS^{e}_{2010}$ (water quality) and the *ECELS* (habitat condition) indexes

		$QAELS^{e}_{2010}$ quality classes				
		I	II	III	IV	V
ECELS quality classes	I	High	Good	Good	Moderate	Poor
	II	Good	Good	Moderate	Moderate	Poor
	III	Good	Moderate	Moderate	Poor	Bad
	IV	Moderate	Moderate	Poor	Poor	Bad
	V	Poor	Poor	Bad	Bad	Bad

Table 7 Percentage of wetlands for each wetland type falling in each quality class (I, high; II, good; III, moderate; IV, poor; V, bad) of water quality ($QAELS^e_{2010}$ index), habitat condition (*ECELS* index) and the resulting ecological status in a selection of 105 wetlands located throughout Catalonia

	N	I	II	III	IV	V
$QAELS^e_{2010}$						
Thalassohaline wetlands	49	8.2	16.3	34.7	34.7	6.1
Permanent freshwater wetlands	43	4.7	34.9	20.9	23.3	16.3
Temporary freshwater wetlands	13	7.7	15.4	46.2	30.8	0.0
ECELS						
Thalassohaline wetlands	49	16.3	32.7	22.4	24.5	4.1
Permanent freshwater wetlands	43	2.3	16.3	37.2	32.6	11.6
Temporary freshwater wetlands	13	30.8	23.1	46.2	0.0	0.0
Ecological status						
Thalassohaline wetlands	49	4.1	14.3	49.0	26.5	6.1
Permanent freshwater wetlands	43	0.0	14.0	37.2	32.6	16.3
Temporary freshwater wetlands	13	7.7	23.1	46.2	23.1	0.0

permanent freshwater wetlands, using them for runoff and irrigation purposes and limiting their overflowing capacity. This affects water quality, but especially habitat condition. On the other hand, the historical fight of humans against temporary freshwater wetlands mainly consists on the burying of these temporary habitats and their substitution by farmlands. Thus, most of them disappear [91–93], but the remaining temporary freshwater wetlands still conserve high ecological standards.

Regarding *ECELS* results in Catalan wetlands, the decomposition of the *ECELS* index in five components allows to distinguish which part of habitat condition is more affected by human pressure (Fig. 8). Scores of the *ECELS* components 2 (human activity) and 5 (hydrophytic vegetation) are those that decrease faster with decreasing habitat condition, while components 3 (water characteristics) and 4 (emergent vegetation) remain unaltered even under intermediate habitat condition. Moreover, when comparing the results by waterbody type, it can be seen that permanent freshwater and thalassohaline wetlands show a gradual pattern of degradation that similarly affects *ECELS* components in both waterbody types. However, the pattern observed from temporary freshwater wetlands was different, and so TF the change from high to good habitat condition in those last wetlands is mainly due to the impoverishment of the hydrophytic vegetation.

$QAELS^e_{2010}$, *EQAT* and *ECELS* indexes are promising tools to evaluate the ecological status in Mediterranean wetlands and can help to provide criteria in the management of these endangered aquatic ecosystems. To recover their ecological functioning and to integrate them within responsible and sustainable human uses in their reception, basins must be a priority in order to ensure the welfare of future generations.

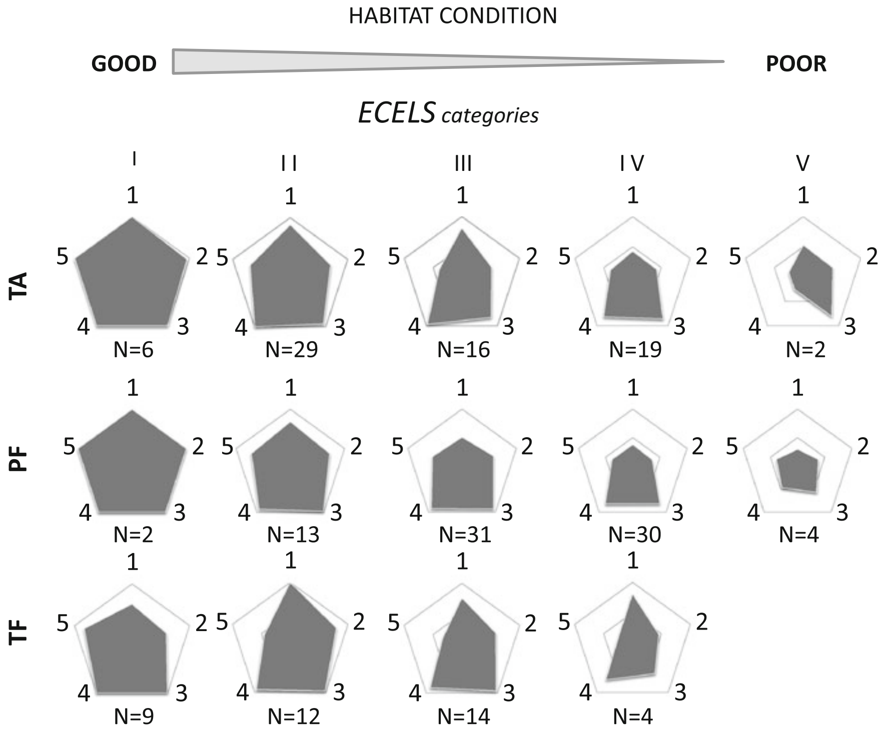

Fig. 8 Values of the *ECELS* components in Catalan wetlands sorted by wetland type (*TA* thalassohaline wetlands, *PF* permanent freshwater wetlands, *TF* temporary freshwater wetlands) and by *ECELS* quality categories (*I*, high; *II*, good; *III*, moderate; *IV*, poor; *V*, bad). Each *pentagon angle* represents a component of the *ECELS* index (*1*, littoral morphology; *2*, human activity; *3*, water characteristics; *4*, emergent vegetation; *5*, hydrophytic vegetation). The width of the *grey area* is proportional to the average value of the score of each component. *N* number of wetlands

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Assessing Ecological Integrity in Large Reservoirs According to the Water Framework Directive

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Abstract In this chapter we review the implementation of the Water Framework Directive in Catalan large reservoirs and the impact of the first Program of Measures on the ecological quality of these water bodies. In this case, the implementation faced a big challenge, resulting from combining a reduced number of water bodies located on highly heterogeneous geological setting and suffering from different and contrasting human impacts. This chapter introduces the proposed methodology, later assesses how it was implemented in a simplified assessment, and finally makes some suggestions for future improvements. In our opinion, a simplified protocol firstly used in Catalan reservoirs for the assessment of ecological potential is a sound, scientific-based methodology that delivers useful information for tailoring the Program of Measures to realistic and achievable objectives. As potential improvements we suggest: (1) the protocol to assess ecological potential should consider the one-out all-out rule for combining the biological and physicochemical quality elements; (2) definition of water body-specific Maximum Ecological Potential situations, using the *Alternative Prague* approach; (3) update the boundaries between levels of ecological potential inside each typology using the best knowledge available from reservoir limnology studies, particularly those published during the last decade; and (4) including the presence of invasive species in the assessment of biological quality.

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Contents

1 Reservoirs in the WFD

The European Water Framework Directive (WFD) 2000/60/EC [1] was approved in December 2000 to protect and improve the quality of European waters. Reservoirs are characterized as *artificial* or *modified* water bodies in this Directive, and pointing to that they were created for economic activities after profound physical modification of the river network and thus have restoration targets different from those defined for the unmodified water bodies [2].

According to the WFD, member states may define surface water bodies as *heavily modified* (HMWB) in the process of drafting river basin management plans. This category was defined to include those water bodies that have been physically altered so that they are substantially changed in character. Alternatively, some water bodies may also be defined as *artificial water bodies* (AWB) if they have been created by human activity [3]. Within this context, physical alterations mean changes to the hydromorphological characteristics of a water body, and a water body that is substantially changed in character is one that has been subject to major long-term changes in its hydromorphology.

The environmental objective for HMWB and for AWB is good ecological potential (GEP), which has to be firstly achieved by 2015, or subsequently by 2021 or 2027. This is in contrast with the more meaningful "good ecological status" objective for the other water body typologies. Nevertheless, GEP is an ecological objective which may be difficult to achieve [4]. However, there is an intrinsic challenge in achieving GEP: establishing an appropriate Maximum Ecological Potential (MEP) for a particular HMWB or AWB. The MEP is considered as the reference conditions for HMWB and is intended to describe the best approximation to a natural aquatic ecosystem that could be achieved given the hydromorphological characteristics that cannot be changed without significant adverse effects on the specified use or the wider environment. There is a controversy about the appropriate criteria to derive MEP, and although the working groups implementing the WFD have tried to give guidance on this, at present little has advanced in terms of understanding what does MEP mean, especially in an ecological context [4].

The Common Implementation Process (CIS) of the WFD has suggested two options to define MEP and GEP that rely on scenario modeling: one based on biological quality elements and the other based on identification of mitigation measures [5]. In the first approach, MEP relates to the values of biological quality elements after all mitigation measures have been implemented that do not have a significant adverse effect on the use of water stored in the HMWB. GEP is defined as only slight changes from those values at MEP. The second alternative, the so-called *Alternative Prague* approach, starts excluding those measures that, in combination, are predicted to deliver only slight ecological improvement. GEP is then defined as the biological values that are expected from implementing the remaining identified (and relevant) mitigation measures. It is argued that the *Prague* approach leads to comparable results as the approach based on biological quality elements, while in the same time, it leaves less room for errors due to predictive modeling [5].

However, there is little guidance based on scientific knowledge on what has to be done with the samples of biological elements. Hence, differences in interpretation, methods, and approaches are common across different European countries [6]. In the best scenario, MEP can be defined using the ecological properties of the closest natural comparable water type, i.e., a natural lake. An alternative is to use an AWB or HMWB of the same type. That would eventually allow for considering "others" than the impacts caused by the hydromorphological changes intrinsically linked to the transformation of a river into a reservoir. However, finding such reference situations in reservoirs is unfeasible in most situations. This particularity of reservoirs should be stressed: although they can be defined as HMWBs, being the river as the parent system, it is evident that using a river as a reference to build MEP would be no sense. However, it is not that obvious that the suggested procedure of using a closest lake to define MEP for a reservoir is equally unreasonable. From an ecological point of view, any comparison between lakes and reservoirs is compromised by the deep differences of ecological functioning between these systems [7].

The difficulties for finding appropriate reference conditions for reservoirs and the ambiguities related to MEP and GEP definition have resulted in undesired side effects during the implementation of the WFD in reservoirs. Most problems stem from the fact that *expert judgment*, in the WFD indicated as a last resort, is frequently the prime mechanism to establish MEP and GEP values. This can be also the result of a lack of sensibility versus the particular ecology of reservoirs and its strong alteration of the river dynamics [8]. As a result, the usefulness of the implementation of the WFD in reservoirs (i.e., the achievement of GEP) is compromised by either its strong dependence on subjective criteria or the use of unreliable metrics developed from natural lake research.

In this chapter we review the implementation of the WFD in Catalan large reservoirs and the impact of this implementation on the first Program of Measures. Several other implementations have been published focusing on streams [9, 10] and coastal waters [11] and water management for agriculture [12]. There are numerous examples of protocols and applications for lakes and other surface waters [13–18]. Nevertheless, implementations published specifically for reservoirs are becoming more frequent [19–23]. In our case, we faced the challenge that only a reduced number of water bodies were available, and those bodies were located in a heterogeneous geological setting and suffered highly contrasting human impacts. The chapter introduces the proposed methodology, how this proposal was implemented in a first simplified version, and suggestions for future improvements.

2 Reservoir Typology in Catalonia

Eutrophication is the main water quality problem in reservoirs due to the larger inputs of nutrients and stronger water-level fluctuations than natural lakes [24, 25]. The MEP of a reservoir will depend to a great extent on the water quality of inflowing river, and in its turn the water quality will depend on the position along the river [26]. Consequently, we suggested an approach that classifies reservoirs into types depending on their position along the river network [27].

The implementation of the WFD for reservoirs in the Catalan River Basin District was based on a specific sampling campaign on 21 large reservoirs located in Catalonia (Fig. 1; Table 1). They were sampled quarterly from summer 2002 to spring 2003. In each reservoir, a sampling point was selected near the dam. Sampling included a wide range of physical, chemical, and biological measurements (temperature, conductivity, dissolved oxygen concentration, nutrient analysis, fish community, phytoplankton and zooplankton communities, etc.). See [27] for further details.

Reservoir typology was established using a collection of variables from system *B* of the WFD: altitude, distance to the sea, volume and the reservoir's catchment area, and geology (using chloride concentration as a convenient proxy). A principal component analysis (PCA) showed that most variables were interdependent. The first PCA axis summarized these correlations displaying a geographical gradient related to altitude and distance to the sea, from lowland reservoirs with high chloride concentration to higher-altitude reservoirs with low chloride. The second PCA axis distinguished two reservoirs of the Ebro River from the rest because of their large basin surface and chloride concentration. Santa Fe, the smallest reservoir, was situated on the opposite side; see [28].

After analyzing the variability along the selected descriptors, we established the boundaries between types by expert judgment, allowing classification of the reservoirs into six types using a dichotomic key (Fig. 2). Escales reservoir was the only member of Type I (large high-altitude reservoirs), whereas Santa Fe was the only one classified as Type II (small high-altitude reservoirs). Discrimination of the two

Fig. 1 Large reservoirs in Catalonia and main rivers. Source: Catalan Water Agency

high-altitude types from the others was accomplished defining a threshold placed at 815 m. Siurana, Foix, and Riudecanyes reservoirs composed Type III, containing small coastal reservoirs (Fig. 2). Type IV was composed by all reservoirs without extreme characteristics along the axes defined by descriptors (i.e., medium-altitude and lowland reservoirs located at least 25 km away from the coast). Chloride concentration over 40 ppm served as a discriminating characteristic between Type IV and the last two types. A threshold value for catchment area of 1,000 km^2 discriminated between Type V (Flix and Ribarroja reservoirs, located in the Ebro River) and Type VI (Sau and Susqueda reservoirs, in the Ter River). All in all, the final classification reflected both the diverse typology of the reservoirs and subjective criteria about the ecological functioning of the systems based on the extensive knowledge available from these systems by the research teams involved in the characterization.

Table 1 Basic morphological characteristics of Catalan reservoirs and ecological potential assessments using the original method proposed by [27] and the simplified implementation

Reservoir	Dam height (m)	Surface area (ha)	Capacity (hm^3)	Ecological potential: original method	Ecological potential: simplified implementation
Boadella	63	363.3	60.2	Moderate	Moderate
Camarasa	103	624	163	Good	Maximum
Canelles	150	1,569	678	Maximum	Maximum
Cavallers	70	47	16	Not assessed	Maximum
El Pasteral	33	34.6	2	Not assessed	Maximum
Escales	125	400	152	Maximum	Maximum
Flix	26	320	11	Maximum	Maximum
Foix	38	67.9	3.74	Poor	Bad
El Catllar	79	326.2	60.4	Not assessed	Poor
Guiamets	47	62	10	Not assessed	Moderate
La Baells	102.3	364.7	109.5	Good	Moderate
La Llosa del Cavall	122.3	300	79.4	Good	Maximum
Margalef	33.2	31.8	3	Not assessed	Good
Oliana	102	443	101	Moderate	Good
Rialp	99	430	402.8	Moderate	Good
Riba-roja	60	2,152	210	Maximum	Good
Riudecanyes	51	40.3	5.3	Good	Good
Sallente	89	31	6.5	Not assessed	Maximum
Sant Antoni	86	927	205	Good	Maximum
Sant Llorenç de Montgai	25	131	10	Good	Maximum
Sant Martí de Tous	34	14.9	1.3	Not assessed	Good
Sant Ponç	59.5	144.5	24.4	Good	Good
Santa Anna	101	768	237	Good	Maximum
Santa Fe	24	6.9	0.8	Maximum	Good
Sau	83	572.8	151.3	Good	Moderate
Siurana	63	85	12	Maximum	Maximum
Susqueda	135	466	233	Good	Good
Terradets	47	330	23	Moderate	Maximum
Vallforners	62	11.4	2.3	Not assessed	Good

3 Assessment of Ecological Potential: Original Proposal

To assess the ecological potential (EP), it is mandatory to establish five classes (maximum, good, moderate, poor, and bad) for all parameters considered in the assessment. The WFD provides extensive guidelines to assess the EP, but only general guidance for defining boundaries between classes [28]. In our case, we used several indexes to define the boundaries between ecological classes. Ten

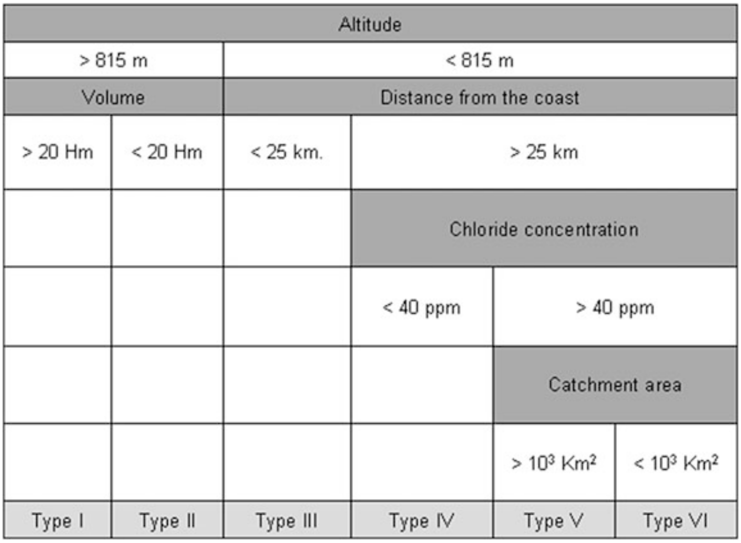

Fig. 2 Classification of reservoir typology in Catalonia

parameters were selected to calculate EP: total chlorophyll-*a* (mg m^{-3}), *Cyanobacteria* chlorophyll-*a* (mg m^{-3}), total and percent catch per unit effort (CPUE) of limnetic and littoral common carp *Cyprinus carpio* [29], percentage of fish with anomalies, Secchi disk depth (m), average percentage of hypolimnetic oxygen concentration, and total phosphorus concentration (mg m^{-3}) in the water column (see Table 2). This set of parameters was expected to comprehensively reflect the physicochemical and biological features of the reservoirs and was used to assess the ecological state of the reservoirs. In the case of nutrients, parameters and boundaries between classes of the Trophic State Index (TSI) [30] and the Organization for Economic Cooperation and Development (OECD) [24] classifications were used. Fish metrics that link the trophic state of the waters with the abundance and species composition of the fish assemblages were also used [29]. The presence of *Cyanobacteria* was considered using the guidelines from the World Health Organization for recreational waters [31], while the Water Quality Index (WQI [32]) was implemented for oxygen conditions.

The lack of unpolluted or pristine reference systems has become one of the emerging problems during the implementation of the WFD [33, 34]. Since reservoirs are one of the most dramatic and irreversible impacts of humans on rivers, the definition of reference systems is not obvious. As a result, some of the boundaries between classes for the indexes mentioned above were modified using expert judgment. Regarding this situation, the choice of a reservoir presenting MEP as a reference for other reservoirs seems acceptable. However, those reference systems to define MEP were not available for two out of the six types defined in the reservoir typology; because with just 21 systems at play and a highly biased distribution toward impacted systems, we could not identify reference systems for Types V and

Table 2 Variables used to assess the ecological potential in Catalan reservoirs and thresholds defining EP levels in the different typologies defined (see Fig. 2). Modified from [27]

Types	Parameters	Maximum	Good	Moderate	Poor	Bad
I, II, III, and IV	Chlorophyll-*a* (mg m^{-3})	0–1	1–2.5	2.5–8	8–25	>25
	Cyanobacteria chlorophyll-*a* (mg m^{-3})	0–0.5	0.5–1	1–5	5–20	>20
	% anomalies in fish	<2%		2–5%	>5%	
	CPUE of littoral carp	<0.005		0.005–0.009	>0.009	
	CPUE of limnetic carp	<0.261		0.261–0.522	>0.522	
	% of littoral carp	<32%		32–64%	>64%	
	% of limnetic carp	<27%		27–53%	>53%	
	Secchi disk depth (m)	>12	12–6	6–3	3–1.5	<1.5
	% hypolimnetic oxygen	100–80	80–60	60–40	40–20	20–0
	Total phosphorus (mg m^{-3})	0–4	4–10	10–35	35–100	>100
V	Chlorophyll-*a* (mg m^{-3})	0–2.5	2.5–10	10–15	15–25	>25
	Cyanobacteria chlorophyll-*a* (mg m^{-3})	0–0.5	0.5–1	1–5	5–20	>20
	% anomalies in fish	<2%		2–5%	>5%	
	CPUE of littoral carp	<0.005		0.005–0.009	>0.009	
	CPUE of limnetic carp	<0.261		0.261–0.522	>0.522	
	% of littoral carp	<32%		32–64%	>64%	
	% of limnetic carp	<27%		27–53%	>53%	
	Secchi disk depth (m)	>8	8–4	4–2	2–1	<1
	% hypolimnetic oxygen	100–75	75–50	50–35	35–20	20–0
	Total phosphorus (mg m^{-3})	0–15	15–25	25–35	35–70	>70
VI	Chlorophyll-*a* (mg m^{-3})	0–5	5–15	15–25	25–50	>50
	Cyanobacteria chlorophyll-*a* (mg m^{-3})	0–0.5	0.5–1	1–5	5–20	>20
	% anomalies in fish	<2%		5–2%	>5%	
	CPUE of littoral carp	<0.005		0.009–0.005	>0.009	
	CPUE of limnetic carp	<0.261		0.522–0.261	>0.522	
	% of littoral carp	<32%		64–32%	>64%	
	% of limnetic carp	<27%		53–27%	>53%	
	Secchi disk depth (m)	>6	6–3	3–2	2–1	<1
	% hypolimnetic oxygen	100–60	60–30	30–15	15–5	5–0
	Total phosphorus (mg m^{-3})	0–16	16–32	32–64	64–128	>128

VI. Therefore, we defined the boundaries between classes for these types by expert judgment, assigning the MEP to the values defined for the GEP. Table 2 illustrates the quality elements and ranges used to assess the ecological status according to the WFD. Note that when calculating the final EP class merging results from the different elements (biological and physicochemical), we always used the most conservative result, i.e., the worst result in terms of final EP assessment was always considered as the outcome [27].

4 Ecological Potential in the Original Sampling

We performed a first evaluation of the ecological potential during year 2003 (i.e., before the period used in the final version of the First Assessment for reporting the EC, 2007–2012). Escales Reservoir, the only Type I reservoir, showed MEP, in correspondence with its definition as a reference system (Table 1). In spite of its headwater position and relatively low chlorophyll values in the oligotrophic range (4–12 mg m^{-3}), the amount of phosphorus released from hypolimnion and sediments (13 mg m^{-3}) during the mixing period produced mesotrophic conditions during the entire year.

Santa Fe Reservoir, the only Type II reservoir, showed high values for both phosphorus (17–35 mg m^{-3}) and chlorophyll-*a* (43–110 mg m^{-3}) because of its dystrophic conditions. *Cyanobacteria* were present in high concentrations (7–11 mg m^{-3} of chlorophyll-*a*), mainly consisting of *Microcystis* sp. and *Gomphosphaeria* sp. However, Santa Fe Reservoir is located in the headwaters of a near-pristine watershed, and those water quality characteristics are typical from dystrophic systems with high inputs from the surrounding deciduous forest. Therefore, Santa Fe was also assigned with a MEP (Table 1).

Type III reservoirs showed the worst EP of all groups, between bad and poor. Foix Reservoir showed the highest values of total phosphorus concentration (250–350 mg m^{-3}) and simultaneous extreme values of chlorophyll-*a* (78–823 mg m^{-3}) and high concentrations of *Cyanobacteria* [35]. The other two reservoirs showed moderate phosphorus concentration (4–50 mg m^{-3}) but eutrophic conditions with high values of chlorophyll-*a* (17–80 mg m^{-3}), resulting from their small size and critical changes in their water levels due to their use for irrigation purposes. All in all, Foix and Riudecanyes showed moderate and GEP, respectively. Siurana Reservoir was the reference system for this type, so it showed MEP (Table 1).

Type IV gathers 12 reservoirs placed on medium-sized rivers, most of them located on adjacent tributaries of the Ebro River. These reservoirs showed moderate and GEP. Most of these reservoirs showed mesotrophic conditions during the year, and only four reservoirs presented eutrophic conditions during part of the year. These four reservoirs showed a moderate EP and should have been the target for restoration measures: Rialb Reservoir, in its initial phases of first filling, and Boadella, Oliana, and Terradets reservoirs because of the poor quality of the inflowing water from tributaries.

Type V systems (Flix and Riba-roja reservoirs) are located at the lower reaches of the Ebro River. The presence of an upstream reservoir (Mequinenza Reservoir, not included in this study, with a volume of 1,533.8 hm^3 and a residence time of 72.5 days) significantly reduces the amount of nutrients. Both reservoirs presented

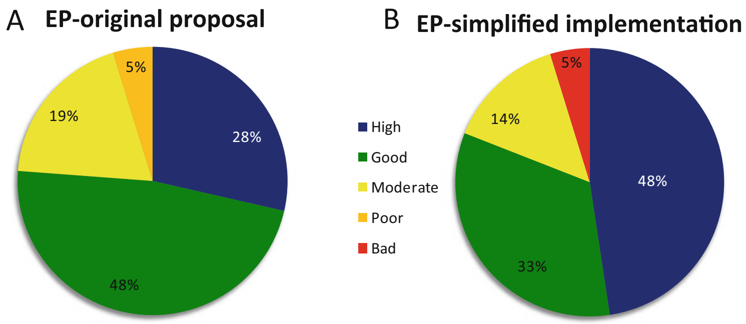

Fig. 3 Ecological potential calculated for the original set of reservoirs using (**a**) the original criteria suggested by [27] and (**b**) the results from a simplified assessment delivered in 2012, considering the same set of reservoirs

mesotrophic conditions during the entire year, with some episodic eutrophic conditions. Reference values were chosen to be in the range of values shown by both reservoirs, considering that their present ecological status is of good quality (i.e., we applied a rather subjective expert judgment). Thus, EP values were between GEP and MEP (Table 1).

Type VI reservoirs are associated with a relatively large river (Ter River). Its watershed suffers from intense human pressures, particularly agriculture and farming, which produce a large amount of diffuse inputs that reach the reservoirs and accumulate in the sediments. Despite the implementation of a sanitation plan that has greatly reduced the nutrient inputs, they are eutrophic or hypereutrophic (78 ± 80 and 62 ± 33 mg m^{-3} total phosphorus). Expert judgment was applied in choosing reference values, with the values of the parameters being quite close to those observed in the reservoirs.

Because of the toxicological relevance of cyanotoxins, chlorophyll-*a* from *Cyanobacteria* was analyzed (81 data from 21 reservoirs) to assess the risk of exceeding 1 μg L^{-1}, the maximum value allowed for GEP. The probabilities of exceeding the limit values of 1 and 5 μg L^{-1} were 19% and 4%, respectively. The six samples over the 5 μg L^{-1} limit were from Santa Fe, Foix, and Riudecanyes reservoirs. During the summer, only Type III reservoirs showed values representing an ecological or human-health risk.

Overall, 28% of the reservoirs were identified as having MEP and 48% as having GEP. The rest of the reservoirs (24%) were below the GEP target (Fig. 3a; Table 1).

5 From Proposal to Simplified Implementation: Ecological Potential Outcomes

The Catalan Water Agency issued a protocol for the assessment of the EP in reservoirs of the Catalan River Basin District, following suggestions contained in [27] and summarized above. This first simplified implementation procedure contained several modifications to tailor it to available monitoring resources and also to test cheaper and quicker procedures. Outcomes from the original and this simplified proposal were compared here; however, it is worth mentioning that the current final implementation (year 2015) is a more complete methodology than the simplified version compared here.

First, the simplified protocol did not include total phosphorus concentration, Secchi disk depth, and parameters related to the fish community. Only total chlorophyll-*a* concentration, chlorophyll-*a* concentration from *Cyanobacteria*, and hypolimnetic dissolved oxygen concentration were considered in the simplified monitoring analysis for reservoirs in Catalonia. Note that the possibility of excluding quality elements from the EP calculation was already considered in early CIS guidance documents and therefore should not be considered as bad practice. Second, the simplified EP value was not equaled to the worst EP value from the different elements (the one-out all-out principle) but computed using the average. The potential impacts of those changes in the protocol on the final EP assessment are discussed later in this section.

The EP was assessed for 30 reservoirs in the simplified assessment, including additional reservoirs beyond the set used to develop the method (Table 1). The simplified assessment identified 46% of the reservoirs in MEP and 32% in GEP (Fig. 4c). This implies that 22% of the reservoirs in Catalonia do not fulfill the target quality requirements (i.e., GEP). Most of the reservoirs identified with moderate EP or less were relatively small reservoirs located near the coast. Remarkably, a large reservoir currently used as one of the main sources for water supply was also identified as having moderate EP (Sau Reservoir), as well as two large reservoirs (Boadella and La Baells reservoirs) used to deal with the seasonal variability of water available for water supply and irrigation in downstream locations (Table 1).

A closer look on the quality elements used to calculate EP gave interesting conclusions. Actually, the physicochemical quality of many samples was identified as bad, while the other half was classified as good (Fig. 4b). However, the biological quality was high in 75% of the reservoirs, and those with moderate or less quality were just 25% of them (Fig. 4a). It becomes clear that the overall EP assignments are more influenced by the biological quality elements than by the physicochemical elements in the simplified implementation.

Unfortunately, we cannot make judgments about non-measured variables, so we can only speculate about the potential impact of the discarded variables in the simplified implementation (Secchi disk depth, total phosphorus concentration, and fish community indexes) on the final EP assignments. However, we can easily check the effect of the criterion to aggregate the biological and physicochemical

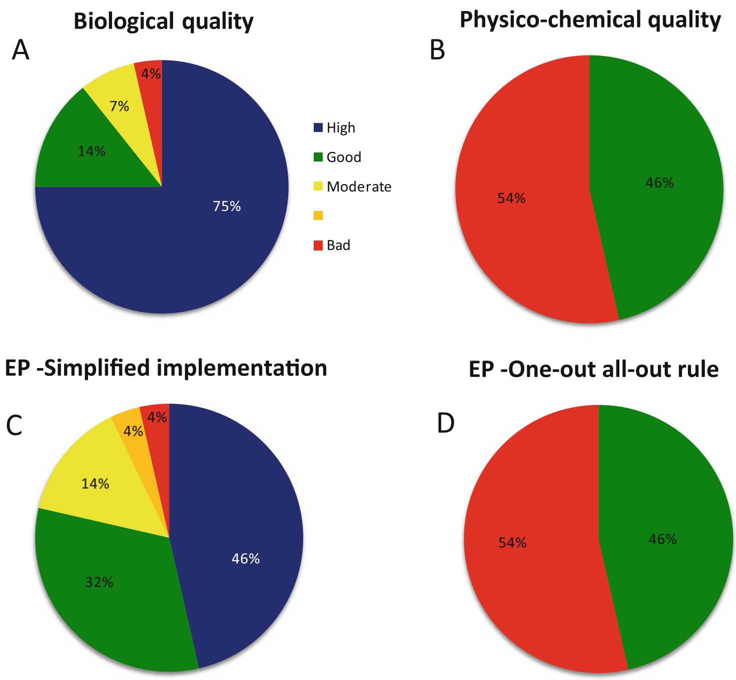

Fig. 4 Results from the simplified assessment for (**a**) the distribution of biological quality scores, (**b**) the physicochemical quality scores, and (**c**) the final ecological potential assignments resulting from the combination of the previous two elements. Additionally, we calculated (**d**) the ecological potential for the same dataset but applying the one-out all-out rule when combining the physico-chemical and the biological quality scores

elements into a single EP class. We compared the EP assignments in the simplified assessment with EP classes computed using the one-out all-out rule when aggregating the biological and physicochemical quality (i.e., we picked the worst result as the final EP). The comparison between the EP assignments in the simplified assessment and using the one-out all-out rule could not be more contrasting (Fig. 4c, d; Table 1). While 46% of the reservoirs still comply with the GEP using the new rule, the rest of them (54%) were classified as having bad EP.

These results stress the fact that choosing the procedures to calculate EP is paramount for the implementation of the WFD in HMWBs and by extension in all water bodies. The suggested procedure by the CIS is to use one-out all-out rules to compute the EP and ecological status (ES), but this is particularly prone to misclassification when a large number of quality elements are included in the assessment. This is not our case, because the number of elements included in the assessment is rather low. Therefore, we have to focus on the ecological meaningfulness of the elements included in the analysis and the appropriate reference conditions established for the different types. The next section is a critical evaluation of the simplified implementation.

6 Critical Evaluation of the Simplified Implementation

In our opinion, the weakest point of the simplified implementation is the fact that it does not consider the one-out all-out rule when combining the biological and physicochemical quality elements to assess the EP. This may imply an overly optimistic assessment (see Figs. 3 and 4). We acknowledge that assuming the one-out all-out rule may in its turn imply an overly pessimistic result, and this fact points to potential problems in the variables selected for the calculation of the biological and physicochemical quality.

The variables selected in the first proposal by [27] were intended to cover the main threats to water quality in HMWBs. Carlson's Trophic State Index (TSI [30]) and the OECD model [36] are the rationale behind the selection of most variables in the original proposal, since cultural eutrophication was considered the main threat. Actually, eutrophication is the most important environmental problem of dammed water [37–39]. A comparison of the results obtained using the Danish method to assign EP categories [18], which is also based on trophic characteristics, gave very similar results [27]. Therefore, the use of variables related to eutrophication seems a good procedure to assign EP categories.

However, both TSI and the OCDE models were first developed for natural lakes, not reservoirs. Indeed, there are problems applying the TSI in reservoirs [40], mainly related to the fact that turbidity in reservoirs can be related to mineral particles, and not to phytoplankton biomass, as assumed in the original study by [30]. Some authors [30, 41–43] also pointed out to the limitation of Secchi disk depth as a trophic state predictor in turbid water bodies like reservoirs. Therefore, the use of transparency (i.e., Secchi disk depth) to track EP in reservoirs should be applied with care. At this respect, the fact that the final implementation did not consider transparency to assess the EP should be considered opportune. In fact, chlorophyll-*a* levels are already a convenient proxy of eutrophication that largely outcompetes transparency. The removal of total phosphorus from the final implementation is not dramatic either: total phosphorus usually covariates with chlorophyll-*a*.

The implementation of the WFD requires the use of fish fauna as a biological quality element. Fish are one of the biological quality elements used to describe the ecological status because they are present in most water bodies, present several qualities to be used as indicators of water quality, occupy several trophic levels, and are considered essential in restoration and management measures. However, many WFD standards are based on the extensive knowledge of Central and Northern Europe aquatic ecosystems [44], but Mediterranean reservoirs have a significantly different functioning compared to natural lakes from Central Europe. In the case of fish fauna, studies on Spanish reservoirs have proved that these type of water bodies present basically introduced species [29], and the fish richness does not seem to be tightly related to EP. This is why Navarro et al. [27] used the percent abundance of cyprinids and variables related to morphological alterations in the suggested methodology. The feeding habit of grubbing through bottom sediments particularly exposes common carp to pollutants accumulated on that compartment of reservoirs, being thus a good bioindicator for chemical pollution [45–47].

In our opinion, the exclusion of fish as a biological quality element to assess EP in reservoirs is not critical, because fish community indicators usually correlate with total phosphorus and other proxies of eutrophication. However, there are several invasive species actively spreading across Spanish reservoirs (e.g., catfish and *Alburnus*), with measurable and significant impacts on water quality [48]. However, the presence of invasive species is not playing a role in the present quality elements defining EP. This is a substantial limitation, because the presence of invasive species may be regarded as one of the fundamental impacts threatening the uses of water. This applies to fishes introduced during the last decades but not to species almost naturalized in the Iberian Peninsula, like *Cyprinus carpio*. Particularly, other invasive organisms like the zebra mussel should be also considered [49]. We suggest that at least a qualitative or semiquantitative monitoring to control modern invasive species should be included in future versions of the methodology to assess EP, especially for those potentially causing strong modifications on the habitats or food webs.

Another potential improvement to assess ecological potential for reservoirs is the use of "tailored" hypolimnetic oxygen thresholds to define the physicochemical quality element, considering other factors than those related with the human impacts. In fact, the simplified methodology considers different thresholds for different reservoir types. However, even considering this, the hypolimnetic oxygen level in reservoirs is highly dependent on climatologic factors that may dramatically vary from year to year [50, 51]. This implies that a reservoir may show contrasting results concerning this parameter irrespective of the pressures and impacts the system suffers. Also, there are reservoirs that may suffer hypolimnetic anoxia promoted by huge inputs of organic matter from the terrestrial ecosystems (e.g., from an extensive deciduous forest). In those cases, hypolimnetic anoxia is not a good proxy of bad EP, because it would be disconnected from human pressures.

All these points converge in a fundamental problem of the simplified methodology: the lack of site-specific MEP references. In our opinion, and similar to the case of rivers in this region, large classification units are not useful for local management because of the environmental heterogeneity typical of Mediterranean watersheds [10]. This is particularly relevant in reservoirs, because they are systems with relatively short water residence times which are strongly modulated by all processes occurring in the upstream watershed. Therefore, the so-called *Alternative Prague* approach, in which MEP values are derived after heuristic scenario assessment, seems the best alternative to improve the current implementation. This approach would require defining MEP values system by system, but it does not necessarily ask for complex dynamic simulation models, because robust empirical load-response models requiring minimal information are available for many parameters. For instance, oxygen levels can be predicted during scenario assessments using the empirical formulations in Marcé et al. [50], while formulations for chlorophyll-*a* in reservoirs are a classical topic resolved many years ago [52]. All these approaches are based on linear regression techniques, so they would be easy to apply and flexible enough to be practical and feasible for heuristic scenario assessment.

7 The Relevance of Reservoir Water Quality on the Program of Measures

A key component of the WFD is the development of river basin management plans which will be reviewed on a six-yearly basis and which set out the actions required within each river basin to achieve set environmental quality objectives. In the case of HMWBs, this is achieving at least GEP. This involves a so-called gap analysis where, for each water body, any discrepancy between its existing status and that required by the Directive is identified. A Program of Measures can then be identified and put in place to achieve the desired goals.

The first Program of Measures for the Catalan River Basin District was delivered on 2010 with the measures to achieve GEP for HMWBs by 2015. A total of 10 out of 30 reservoirs were identified as not compliant with the required objective (GEP) in 2009, and the objective of the Program of Measures is to reduce the number of noncompliant systems to 2 in 2015 (corresponding to El Catllar and Foix reservoirs).

As for the concrete measures present in the Program of Measures that concern reservoirs, most of them refer to management strategies to ensure appropriate environmental flows, downstream reservoirs, and sufficient sediment load to receiving rivers to maintain a correct morpho-sedimentary dynamics. However, the Program of Measures did not include many actions explicitly devoted to improve the ecological potential of those reservoirs which were not compliant with the GEP objective in 2009. The only highlighted measure unequivocally pointing to the ecological potential of a reservoir is the remediation program to remove contaminated sediments from Flix Reservoir. This is a huge remediation program with a budget from the Spanish Government amounting to ca. 155 million euro, and the main goal is to remove from the reservoir industrial-contaminated sediments with several priority substances.

Although we acknowledge that any measure taken to improve the upstream river water bodies will ultimately impact the reservoir as well, this should not be considered as a guaranteed outcome of the Program of Measures. Reservoirs have a strong tendency to keep eutrophication conditions despite remediation measures due to the lasting influence of sediments on water quality.

Another relevant aspect of the Program of Measures as far as it concerns reservoirs is the extensive space devoted to invasive species. Both, zebra mussel (*Dreissena polymorpha*) and fish introductions are considered two of the main threats to the EP in reservoirs along the document, with particular protocols and prevention measures defined. This is reflected by the fact that the Control and

Surveillance Program of the Catalan Water Agency already considers early warning systems for the detection of new invasions by these species. However, this vividly contrasts with the fact that the presence of invasive species is not considered in the current assessment of EP in reservoirs and that all fish community elements have been removed from the biological quality element to assess EP.

8 Final Remarks

The implementation of the WFD across Europe has been the magic bullet to put freshwater quality and ecosystem health at the forefront of policy priorities during the last decade. As an ambitious Directive, its implementation is an enormous scientific and policy challenge that has boosted, and will keep pushing, basic and applied research in Europe. This implies that the scientific-based protocols for the assessments and the overall strategy of the concrete policies steaming from DMA implementation have been modified and will continue changing during at least the next decade. Actually, the monitoring programs have already provided enough data to elucidate whether the EP and ES boundaries and water body types proposed in the protocols work in accordance with the spirit of the WFD.

In our opinion, the protocol for the assessment of EP in Catalan reservoirs is a sound, scientific-based methodology that delivers useful information for tailoring the Program of Measures to realistic objectives. However, it is evident that some improvements are still possible. We suggest the following modifications for future revisions of the protocol:

- The protocol to assess EP should consider the one-out all-out rule for combining the biological and physicochemical quality elements.
- Define water body-specific MEP situations, using the *Alternative Prague approach*.
- Update the boundaries between levels of EP inside each typology using the best knowledge available from reservoir limnology studies, particularly those published during the last decade.
- Include the presence of invasive species in the assessment of biological quality.
- The most recent studies disentangling the contribution of both the climatic change and the human-derived impacts on the water quality of reservoirs may allow for a more precise threshold establishment for certain EP metrics (e.g., oxygen levels).

We are confident that these changes would facilitate the definition of concrete actions in forthcoming Program of Measures, because the EP objectives would be tailored to already defined and realistic management options. And last but not the least, it would improve the EP of our water bodies, which is the final aim of the WFD.

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Hydromorphological Methodologies to Assess Ecological Status in Mediterranean Rivers: Applied Approach to the Catalan River Basin District

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Abstract Methodologies currently used to assess hydromorphological features in Mediterranean rivers are reviewed in this chapter. Most relevant methodologies developed across Europe in compliance with WFD (Water Framework Directive) are also analyzed, along with their adaptations to different spatial scales from European, national to regional scales. We also present those hydromorphological protocols that have been developed, used and tested in the Catalan River Basin District, within the framework of monitoring programmes under the requirements of the WFD. The Catalan Water Agency developed a comprehensive protocol to assess hydromorphological conditions in Catalan watersheds, named HIDRI, which assesses and combines hydrological alteration, river continuity and morphological conditions. HIDRI is a compiled protocol based on different metrics and includes large information at river catchment scale.

This chapter also introduces challenges and opportunities in using hydromorphological information for river management. Considerations for an extensive use of hydromorphology assessment in Mediterranean rivers are presented as well as those recommendations to be included in River Basin Management Plans and in the Programme of Measures to achieve good ecological status according to the WFD objectives.

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Keywords Catalan River basin district, Eco-hydromorphology, HIDRI protocol, Hydromorphological assessment, River basin management plan, Water framework directive

Contents

Abbreviations

ACA	Catalan water agency (in Catalan: *Agència Catalana de l'Aigua*)
CEN	A guidance standard for assessing the hydromorphological features of rivers (2002). CEN-TC 230/WG 2/TG 5: N32.
CRBD	Catalan river basin district
EU	European Union
HIDRI	Hydromorphological quality index used in Catalonia (in Catalan: *Protocol d'avaluació de la qualitat HIDromorfològica dels RIus*)
HYMO	Hydromorphology
IHA	Index of hydrological alteration
IHF	River habitat index (in Spanish: *Índice del Hábitat Fluvial*)
QBR	Riparian forest quality index (in Catalan: *Qualitat del Bosc de Ribera*)
RBMP	River basin management plan
WB	Water body
WFD	Water Framework Directive (2000/60/EC)

1 Introduction

The EU Water Framework Directive [1], hereafter WFD, reinforced the need for a more holistic view of river management by introducing the concept of ecological status assessment, mainly based on biological quality elements (i.e. macrophytes, phytobenthos, invertebrates, fish) which naturally inhabit those aquatic ecosystems. This directive also requires that the hydromorphological and physicochemical conditions should allow the appropriate structure and functioning of such communities in order to achieve the good ecological status. Within the requirements of the WFD, the assessment of hydromorphology features (hereafter HYMO) includes the assessment of several related variables such as flow regime, sediment transport, river continuity, geomorphology and lateral channel mobility. HYMO embraces hydrology, geomorphology and ecology and has generated a new understanding of physical processes within river management and river restoration strategies [2]. Shortly after the adoption of the WFD, the European Commission launched a standard guidance (CEN) in order to homogenize methods on field data collection and subsequent data handling for the assessment of HYMO [3]. This guidance is particularly focused on the characterization of hydromorphological changes due to human pressures, and it represents one of the largest efforts to standardize the monitoring of physical characteristics of river habitats. It does not provide specific methods but establishes relevant key elements so as to characterize and evaluate HYMO quality. Concepts provided by this guidance have been developed after recent research works on habitat structure and biological communities [4]. HYMO status assessment has been identified to be a critical element for ecological status improvement of aquatic ecosystems in Europe; and therefore, suitable protocols for HYMO quality assessment are required to better analyse ecosystem alterations and to identify problems to be solved. Nevertheless, procedures to obtain accurate data from habitats and other hydromorphological parameters are complex and have not been properly implemented so far. Appropriate protocols for monitoring HYMO parameters and for data interpretation are still under development and discussion in many countries, and furthermore, in Mediterranean aquatic ecosystems, additional issues have to be addressed such as water scarcity and the presence of temporary and/or intermittent water bodies.

It is well known that physical heterogeneity favours biological communities and thus enhances biological diversity in rivers [4]. However, few studies have documented the relationship between habitat degradation and its impact on macroinvertebrate community [5] or the positive effects of restoration projects on aquatic biota. There is a need of further scientific studies, technical applications and consensus on the integration of physical habitat and biological descriptors. Some of the difficulties for this integration lie in the fact that different spatial scales are relevant to different biological communities; therefore, assessment procedures ideally should provide information on pressures that degrade habitat at each of these spatial scales [6]. Some other difficulties lie in the fact that multiple pressures influence freshwater communities and these might act synergistically [7]. Most common indices applied so far, when describing physical stream characteristics, do not include some relevant parameters such as hydraulic geometry or geomorphic processes along the stream corridor. For instance, flow resistance and water velocity, which are related to sediment transport and channel morphology, are often not considered; however, they have significant consequences on habitat availability for biological communities [8]. Hydrodynamics play an important role in regulating biological functions [9], and even simple hydraulic variables like the Froude or the Reynolds number may explain fish population structure in rivers [10].

A wide variety of methodologies have been proposed for the characterization of river habitat in order to assess ecological status according to the WFD. However, monitoring physical characteristics of river habitats lacks a mid- to long-term standardized methodology. A homogeneous procedure for measuring water quality which combines biological and chemical elements has been widely applied so far [11], contrasting with scarce well-established methods for HYMO monitoring, and each country has developed its own HYMO methodologies to comply with WFD requirements. This chapter mainly introduces the challenges in assessing HYMO conditions in Mediterranean rivers and its role in river management plans, as well as the state of the art of HYMO assessment in Spain, specially focused on Catalonia, and the HIDRI protocol that the Catalan Water Agency (ACA) launched in 2006 to assess the HYMO quality in Catalan rivers.

2 Methods for the Assessment of Hydromorphological Quality According to the WFD

2.1 Most Relevant HYMO Quality Assessment Methods Used in Europe

On one hand, most of the existing methods on HYMO assessment are designed to gather information at local scale (reach or sampling site) and require field surveys. Examples of these current methodologies are the River Habitat Index (IHF) [12] or the Riparian Forest Quality Index (QBR) [13]. On the other hand, geomorphological-oriented methods include physical features as well as long-term temporal scale processes and the need of data on large-scale variables. Examples of the latter are the River Styles Framework [14], the SYRAH (*Système Relationnel d'Audit de l'Hydromorphologie des Cours d'Eau*) [15] and the Index for Hydrogeomorphological assessment (IHG, named originally *Indice Hidrogeomorfológico*) [16], which are based on hydrogeomorphological dynamics and consider the functional quality of fluvial systems, the channel quality and the quality of river banks. Among those methodologies applied by EU state members (Table 1), there are some relevant methods that have been used before the development of CEN standards, and in fact, they are the basis of this guidance standard: the field survey method of the Landarbeitsgemeinshaft Wasser (LAWA-vor-Ort) from Germany [21], the River Habitat Survey from UK [20] and the Systeme d'Evaluation de la Qualité du Milieu Physique (SEQ-MP) from France [32]; all of them are examples of integrated protocols.

Table 1 summarizes the main HYMO methods and/or assessment criteria compiled by a project funded by the European Commission, named REFORM (REStoring rivers FOR effective catchment Management) whose objective is to improve the knowledge on HYMO methodologies for the implementation of the WFD (reform rivers restoration wiki).

Table 1 Inventory of most relevant and recent hydromorphological (HYMO) methods and/or assessment criteria applied in European countries following the requirements of the WFD [7]

Methodology	Country	Reference	Application
Guidelines for assessing the HYMO status of running waters	Austria	[17]	
HEM	Czech Republic	[18]	
DSHI	Denmark	[19]	
RHS; EFI	England, Wales	[20]	Commonly used since 2000. EFI has been recently developed by EA
CarHyCe; Syrah& Aurah-CE; ROE &ICE	France	[15]	CarHyCe is used as the official one and Syrah-ce, Aurah-ce and ROE & ICE to comply with WFD requirements
LAWA-FS; LAWA-OS	Germany	[21]	LAWA-FS is the most commonly used, but LAWA-OS was selected for the River Basin District Analysis 2004
RHAT	Rep. of Ireland	[22]	Developed to comply with WFD
MQI; IARI; CARAVAGGIO	Italy	[23, 24]	MQI, IARI and CARAVAGGIO for the overall HYMO assessment and CARAVAGGIO for reference sites
Method to assess HYMO changes	Latvia	–	Used in the definition of HYMO changes in river basin district projects
Handboek HYMO	The Netherlands	[25]	It has not been officially selected
MHR	Poland	[26]	Officially approved for the HYMO assessment of rivers
Adaptation of RHS	Portugal	[27]	In accordance with the WFD requirements and adopted by Portuguese Water Authorities
Criteria and parameters for assessment of HYMO significant pressures	Romania	–	For the designation of HMWB
MImAS	Scotland	[28]	Proposal tool to support the assessment and monitoring of the ecological status of rivers
HAP-SR	Slovakia	[29]	Method proposed for the assessment of ecological status of rivers in the Slovak Republic
SIHM	Slovenia	[30]	National method for the implementation of the WFD
IHF; QBR	Spain	[12, 13]	Both methods are widely used by Water Agencies for HYMO assessment under WFD requirements. They are used at a local scale

(continued)

Table 1 (continued)

Methodology	Country	Reference	Application
Assessment criteria for HYMO quality elements; Biotope Map	Sweden	[31]	Criteria for the assessment of HYMO quality elements to assess good and high ecological status. The Biotope Map is the most used field method to collect environmental variables

2.2 HYMO Quality Assessment Methods Applied in Mediterranean Rivers

Methods in Table 1 consider features and processes for the assessment of HYMO quality mainly considering permanent rivers. In contrast, many Mediterranean rivers suffer water scarcity and alterations in their natural flow regime, and in some cases, these pressures are worsened by the presence of large reservoirs used as water storage for irrigation and/or drinking. Seasonal or intermittent rivers in Mediterranean areas require adapted methodologies, since protocols developed up to now for HYMO assessment have been specifically designed for permanent water bodies.

Mediterranean rivers have a high temporal variability, with dry and wet periods, making it difficult to characterize these aquatic ecosystems. In Mediterranean areas, the hydrological regime is a key element that determines community composition [33, 34] and its response to the annual and seasonal hydrological variability [35]. Numerous studies have revealed the peculiarities of Mediterranean and temporary streams where temporal changes in the composition of the invertebrate community are related to flow regime variation [36]. Thus, reference conditions might change between dry and wet periods and after extreme hydrological events in the same river type, which complicates the HYMO quality assessment. Also, natural hydromorphological processes associated with intermittent Mediterranean streams can be modified by human pressures. In these cases, flow variability increases difficulties of identifying and assessing hydromorphological features such as bankfull characteristics, erosion and deposition shapes, substrate type, macrophyte growth, riparian community structure, among others [27]. Moreover, low-flow situations result in water quality degradation, as a confounding factor.

Some of HYMO standardized assessment protocols recently used by some Mediterranean countries have been developed from the abovementioned methodologies, with some adaptations as HCI or Caravaggio (Table 2). These methodologies assess physical habitats alone, without taking into account physical processes. In this sense, changes in physical habitat do not allow a sufficient understanding of the causes of pressure response. A comparison among these main Mediterranean HYMO assessment methodologies is presented in Table 3 [7], which is based on the analyses of the following items:

Table 2 A selection of methods for the assessment of physical habitats in Mediterranean rivers

Methodology	Country	Application
1. HCI (Adaptation of RHS Portugal) [27]	Portugal	All water bodies
2. Caravaggio [23]	Italy	All water bodies
3. CarHyCe [15]	France	All water bodies
4. HIDRI protocol [37]	Spain (Catalonia)	All water bodies

Table 3 Comparison of main characteristics within selected methodologies [7]

Item	HCI	Caravaggio	CarHyCe	HIDRI protocol
Data collection				
Complemented tools (maps, remote sensing, habitat models, etc.)	No	No	No	Yes
Rapid field assessment	Yes	Yes	No	Yes
Existing database	Yes	Yes	Yes	Yes
Spatial scale				
Hierarchical scale	Survey unit	Survey unit	Survey unit	Reach
Longitudinal scale	Fixed length	Fixed length	Length vs. width	Variable length
Lateral scale	Channel, riparian zone, floodplain	Channel, riparian zone, floodplain	Channel and riparian zone	Channel, riparian zone, floodplain
Temporal scale				
Present	Yes	Yes	Yes	Yes
Historical	No	No	No	No
Selected features assessed:				
Longitudinal continuity	Yes	Yes	Yes	Yes
Lateral continuity	Yes	Yes	Yes	Yes
Bank erosion/stability	Yes	Yes	Yes	No
Channel adjustments	Yes	No	No	No
Vertical continuity	Yes	Yes	Yes	Yes
Habitat complexity	Yes	Yes	No	Yes

1. Data collection methodology and quality of data provided: rapid field assessment or a complex field survey and existing or new database
2. Spatial scale: hierarchical, longitudinal and lateral spatial scale
3. Temporal scale: present or historical scale
4. Type of assessment provided, among a range of qualitative or quantitative information about the condition of a set of river habitat characteristics: longitudinal, vertical and lateral continuity, bank erosion and stability, channel adjustment or others

2.3 HYMO Quality Assessment in Mediterranean Spanish Rivers

Nowadays, there are several indices in use throughout the Iberian Peninsula regarding the assessment of physical river habitats. Examples of these are the River Habitat Index (IHF, named originally *Indice de Hábitat Fluvial* in Spanish) that evaluates river bed habitat heterogeneity based on several physical variables [12], the Riparian Forest Evaluation Index (RFV) [38], the Riparian Forest Quality Index (QBR) [13] and the Riparian Quality Index (RQI) [39]; the last three assess the riparian forest quality and the river channel morphology. However, all these indices only evaluate one or few elements among those required by the WFD to analyse HYMO quality (hydrological regime, continuity and morphology). The QBR index consists of four blocks that take into account different eco-hydromorphological conditions: (i) block 1, total riparian cover; (ii) block 2, cover structure; (iii) block 3, cover quality; and (iv) block 4, river channel naturalness. Nevertheless, hydrological alterations and river continuity are not specifically considered in the QBR index, and additional measurements are required. The Spanish Water Authorities usually use the IHF index together with the QBR index to assess HYMO quality, whose reference values and objectives have been determined for each river type [40]. In Catalonia, a set of metrics were integrated into a more complex protocol, named HIDRI (in Catalan: *Protocol d'avaluació de la qualitat hidromorfològica dels rius*) [37] that assesses all WFD HYMO features (hydrological regime, river continuity and morphological conditions) by using QBR index and others. This protocol will be widely explained in next section of this chapter.

A study was undertaken from 2009 to 2011 [41] in order to compare the official methods used in Spain (IHF and QBR), with the River Habitat Survey (RHS), a method widely used in central and north European watersheds. Within this study, the Habitat Quality Assessment Index (HQA) and the Habitat Modification Score (HMS), both resulting from the RHS [20], were calculated and compared with the regionally widely implemented indices (IHF and QBR) in several river sampling sites located in the Spanish Mediterranean area. The four indices were assessed in a total of 190 sites across 19 Mediterranean river types (Fig. 1). Five reference sites and five sites with different degrees of disturbance were selected for each river type in order to analyse a wider range of hydromorphological conditions. Stream flow was also measured at each site using the Catalan Water Agency procedures provided by the HIDRI protocol [37]. Flow data allowed to identify survey hydrological conditions for every river reach and thus to help interpret results of HYMO indices as well as biological indices also applied in this study.

Results showed that the QBR index is less conditioned by flow conditions because it is based on the assessment of the riparian community, more resilient to flow changes. The IHF index assesses characteristics of different river bed habitats (frequency of rapids, water velocity and depth) as well as the cover of different types of aquatic vegetation; thus, the use of this index is recommended during periods of low flow. However, IHF values might be underestimated in extremely

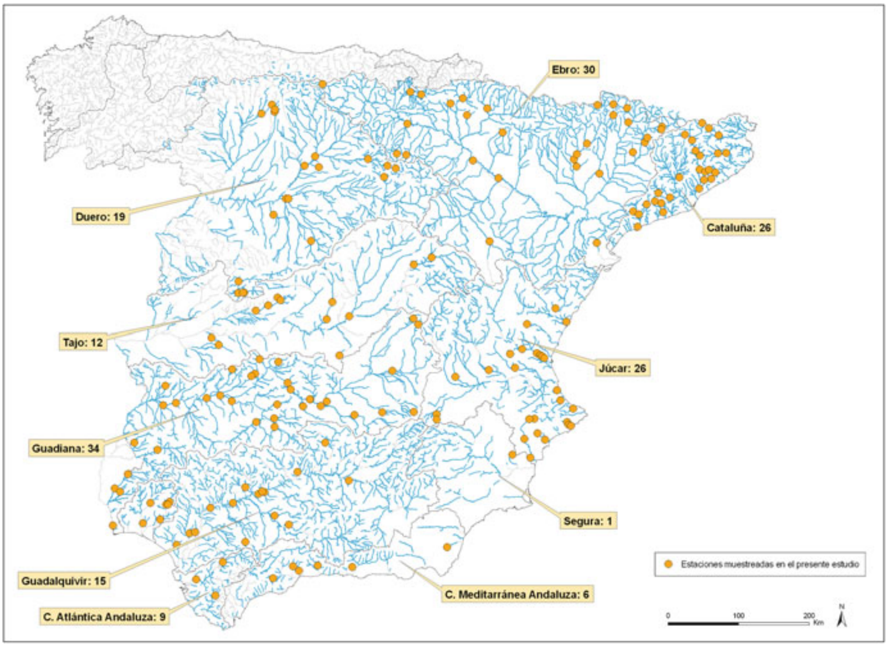

Fig. 1 Location of sampling sites for HYMO assessment in Mediterranean Spanish basins [41]

low-flow conditions and overestimated in high-flow conditions [12]. The HMS scores artificial modification of the river channel morphology and thus elements not directly related to stream flow. The HQA scores natural features of the channel such as bars, diversity of channel substratum, flow types, in-channel vegetation and also the extent of bank-top trees and the extent of natural land use adjacent to the river. Therefore, in extreme low-flow situations, it is likely that, for instance, the number of alluvial bars and other natural features are overestimated, and other elements such as flow types and mesohabitats are underestimated. All four HYMO indices were compared, including QBR blocks 1 and 4 (QBR1, the riparian zone cover, and QBR4, the degree of channel modification), and correlated to a pressure gradient that was previously calculated taking into account all studied sites. This pressure gradient was mainly related to physicochemical alterations and urban and agricultural land use [42]. All of HYMO indices were significantly correlated (p <0.0001) with this pressure gradient (Table 4), and all indices were negatively correlated with this pressure gradient, except for HMS, which correlated positively, since it reflects the degree of HYMO modifications.

Spearman correlation (Rs) and Pearson correlation (r) values ranged between 0.46 and 0.62 (absolute value). The QBR index had the highest linear correlation, also in absolute value, with the pressure gradient ($r = -071$) and the highest percentage of variance explained (51%) by the pressure gradient. All HYMO indices (HQA, HMS, IHF and QBR) were significantly correlated with each other ($p < 0.001$) (Table 5). It is worth noting that the HQA does not scores the

Table 4 Correlation coefficients (r = Pearson; rs = Spearman) between hydromorphological indices and the human pressure gradient; p = level of significance; n = number of sites in each group; R2 of the trend line

	IHF	*QBR4	*QBR1	QBR	HMS	HQA
r	−0,50	−0,49	−0,61	−0,71	0,46	−0,61
p	$p < 0{,}0001$	$p < 0{,}0001$	$p < 0{,}0001$	$p < 0{,}0001$	$p < 0{,}0001$	$p < 0{,}0001$
rs	−0,48	−0,48	−0,62	−0,71	0,49	−0,59
p	$p < 0{,}0001$	$p < 0{,}0001$	$p < 0{,}0001$	$p < 0{,}0001$	$p < 0{,}0001$	$p < 0{,}0001$
n	176	176	174	174	171	171
R^2	0,25	0,24	0,38	0,51	0,22	0,38

[a]QBR blocks 1 and 4 were also included

Table 5 Correlation values among all HYMO indices assessed. All correlations are significant ($p < 0.001$), and the higher correlations ($>|0.5|$) are given in bold. *QBR blocks 1 and 4 were also included

	IHF	QBR4*	QBR1*	QBR	HMS	HQA
IHF						
QBR4	0,324					
QBR1	0,390	**0,520**				
QBR	0,469	**0,701**	**0,826**			
HMS	−0,317	**−0,706**	**−0,578**	**−0,677**		
HQA	0,422	0,424	0,416	**0,592**	**−0,544**	

percentage of cover in the channel (IHF) neither collects information on the vegetation cover in the riparian zone (QBR). A part from this, the only index that scores degradation elements in rivers (HMS) is inversely correlated with the other indices. This means that for higher values of HMS, low scores for IHF, QBR and HQA indices are obtained. The QBR index and blocks 1 and 4 from QBR index showed the highest correlation with the other indices (>0.5). QBR block 4 (channel modification) is more correlated with the HMS values (rs = −0.706; $p < 0.001$), than with QBR block 1 (riparian cover), or total QBR score. Both QBR block 4 and the HMS score the presence of artificial channel infrastructures and modifications of river banks.

The interpretation of the scores obtained in this study for the different HYMO indices should consider both stream flow conditions at the moment as well as the interannual variability. In this sense, the following issues should be taken into consideration:

- Habitat quality in Mediterranean rivers improves with increased flow conditions. More diversity in habitat types is observed [43], sediment inclusion decreases with higher flows [35], and more leaves and branches are dragged with flood events [12].
- The QBR at given sampling site is less influenced by seasonal variability as this index assesses riparian cover as well as shrub and perennial vegetation structure

[36]. However, in those types of Mediterranean rivers exposed to hydrological stress, a typical riparian forest is unlikely to progress [43].

3 The HIDRI Protocol, a Comprehensive HYMO Method Applied in Catalan Rivers

The Catalan Water Agency (ACA) developed a protocol to analyse the three HYMO quality elements listed in the WFD: hydrological flow regime, river continuity and morphological conditions, named HIDRI protocol [37]. This protocol combines all these quality elements and metrics in order to give a final value of hydromorphological quality, including the QBR index mentioned above. HIDRI protocol has been applied in all river water bodies (248 WB) of the Catalan River Basin District (Fig. 2) according to the monitoring programme planning. Each river water body has a minimum of one sampling site to evaluate HYMO conditions as well as biological and physicochemical parameters.

While the physicochemical and biological quality is evaluated at one site considered as representative of the water body, the HYMO quality assessment requires incorporating protocols that evaluate the whole WB by assessing the degree of HYMO deviation from reference conditions along WB. This protocol is based on field survey, but it has the potential to include large-scale information and can be implemented using much of the existing information in the River Basin District through its own monitoring and control networks. The HYMO assessment is applied, at least, once every 6 years. The inclusion of HYMO quality in the ecological status assessment of WB contributes to an integrated river management planning by taking into account river continuity, morphological conditions and flow regime alteration according to the WFD.

The assessment of river continuity refers to the longitudinal connectivity of rivers, in terms of water and sediment transport from the source to the mouth and in terms of mobility of biological communities, which can be affected by the presence of obstacles such as weirs and dams and by flow regime alteration. The existence of obstacles across the channel has important ecological consequences and is considered one of the main causes of the decline of many species of fish, especially those that migrate to complete their life cycle. Lateral connectivity should be also considered through the analysis of morphological conditions, and it refers to the connection of the river banks with adjacent ecosystems. This connectivity can be reduced by the fragmentation of riparian forest, by artificial land uses and by the presence of barriers or river channelling. Within the assessment of morphological conditions, in-stream characteristics are also evaluated (i.e. structure and substrate of the river bed) as well as the riparian zone; both of them determine the structure and processes of the biological communities of the river channel and the relationship with other dependent ecosystems.

Fig. 2 Location of the Catalan River Basin District

The HIDRI protocol summarizes all abovementioned characteristics and quality elements (Table 6), and it has been applied in the Catalan rivers over the last decade in order to obtain data which was used to develop a special report on the hydromorphological quality of rivers in the Catalan River Basin District [45].

3.1 Hydrological Flow Regime

The hydrological flow regime alteration is calculated combining the water withdrawal degree analysis (WW), the environmental flow compliance (EFC) and the index of hydrological alteration (IHA) according to the HIDRI protocol.

Table 6 Parameters and metrics considered by the HIDRI protocol (used in Catalan rivers)

Elements	Parameters	Metrics
Hydrological regime	Water withdrawal	Water withdrawal degree at water body level (theoretical evaluation)
	Environmental flow compliance	Relation between measured flow and environmental flow objective (real valuation from punctual measurement)
	Alteration of hydrological regime	Indicators of hydrologic alteration (IAHRIS) in reservoirs (Deviation from the natural regime)
River continuity	Longitudinal continuity of the river channel	Obstacle density in water bodies
		Permeability evaluation of barriers (river connectivity index-ICF) [44]
Morphological conditions	Structure and substrate of the bed. Lateral continuity	Degree of channelling
	Structure of the riparian zone	Naturalness of river banks based on land use analysis
		Riparian forest quality (QBR) [13]

3.1.1 Water Withdrawal Degree (WW)

This metric takes into account the number of water abstractions or registered water diversion sites, the usable water volume and the available water flow. It must be evaluated for each water withdrawal individually (WW_{ind}) and later integrated for the WB evaluation (WW_{WB}). The theoretical flow downstream of a water withdrawal site is calculated using registered data (maximum legal flow withdrawal and environmental flow if established); ordinary flows from gauging stations or simulated natural flow regime is considered as well as the environmental flow regime target for each site.

The WW_{ind} is calculated on monthly basis, by comparing the flow downstream the abstraction site (Q_d) with the highest value between the environmental flow (as a reference) (Q_{env}) and the upstream flow (Q_{up}). This comparison follows this logic:

$$\text{if } Q_{up} > Q_{env} \rightarrow WW_{ind} = Q_d / Q_{up};$$
$$\text{if } Q_{up} \leq Q_{env} \rightarrow WW_{ind} = Q_d / Q_{env} \text{ with a maximum value of 1.}$$

In those WB without identified water uses, quality will be estimated as very good, except in those cases in which flow regime alterations come from upstream reaches.

It is important to distinguish a non-consumptive water diversion from a consumptive water diversion (e.g. for irrigation). Consumptive water diversion affects the entire river from the abstraction site, while a non-consumptive diversion, as the derivation for hydroelectric use, affects the river stretch between the water abstraction site and water return site. Therefore, the river length affected by a diversion

Table 7 Water withdrawal quality assessment according to the water withdrawal degree (WW). Same intervals are used for the compliance of environmental flows (EFC)

Result of WW_{WB}	WB quality
WB without withdrawals	High
$WW > 0.9$	Good
$0.6 < WW > 0.9$	Moderate
$0.3 < WW > 0.6$	Poor
$WW < 0.3$	Bad

must be considered when calculating the water withdrawal degree for a given WB as follows:

$$WW_{WB} = (\text{WB length} - \Sigma\, ((1 - WW_{ind}) \times \text{river length affected by diversion})) \,/\, \text{WB length}.$$

Once values of WW_{WB} are calculated, a quality level is assigned to each water body according to the Table 7 criteria.

3.1.2 Environmental Flow Compliance (EFC)

The environmental flow compliance is evaluated in those water bodies subject to water abstraction, transfer or diversion, by comparing real flow data (measured from gauging stations or estimated from water derivation sites) with an environmental flow as a target. The environmental flow compliance degree is calculated monthly for each withdrawal as follows (ind = individual or w = weighted):

$$EFC_{ind} = \text{measured flow } (Q_m)/\text{environmental flow reference } (Q_{env})$$

The annual average is calculated from these monthly data, and for those cases, with data available from several years, the annual average is estimated from this longer period. If there is no real flow data information, the water withdrawal site remains unrated as well as its corresponding WB.

The length of the river segment affected by water diversions for hydroelectric uses is considered as in the previous section.

$$EFC_w = EFC_{ind} \times (\text{river length affected by diversion} \,/\, \text{WB length})$$

The final evaluation for each WB corresponds to the sum of all individual and/or weighted ECF. There are five levels of quality for the assessment of environmental flow compliance, with the same intervals as for the water withdrawal degree (Table 7).

3.1.3 Index of Hydrological Alteration

The US Nature Conservancy developed a method known as Indicators of Hydrological Alteration (IHA) [46–48] for assessing the degree of hydrological alteration attributable to human pressures. The method is based on statistical analyses of 33 hydrological items representing five streamflow characteristics that play a major role in determining the nature of aquatic and riparian ecosystems. These indicators have been adapted in Spain through the "IAHRIS" method [49]. Both offer free software that allow calculating, with daily or monthly flow data, parameters to characterize the hydrological regime as well as indices to assess the degree of hydrological alteration and criteria for the assignment of heavily altered WB and to assess environmental flow scenarios.

This method (IHA) has been applied in Catalonia to those WB affected by reservoirs with high capacity of regulation, and the following considerations have been taken into account:

- In those WB that are subject to different types of hydrological regime alteration, only the most significant one has been characterized.
- The degree of hydrological regime alteration may vary depending on the time series used. Time span should be similar and as recent as possible for all WB, so that results might be comparable. The period of flow data required in IAHRIS is 15 years.
- The IHA is applied at specific sites, mainly in large reservoirs. Results might be extrapolated to downstream water bodies affected by this pressure unless there other significant changes in their hydrological conditions.

The quality level according to the hydrological regime for each WB is obtained from the combination of the three parameters mentioned above (Tables 8 and 9). The combination criteria are conservative, thus good quality is achieved when there is no significant water abstraction, transfer, diversion nor water flow regulation.

3.2 River Continuity

The River Connectivity Index (ICF) [44] used by the HIDRI protocol is based on the assessment of barriers as well as crossing devices for aquatic biota, if present, with the potential fish fauna ability to surmount them. This index takes into account the swimming and/or jumping ability of all fish native species that are potentially present in the river reach; and it differentiates whether the infrastructure might be crossable for all species, only for some species or impassable. Results are classified into five categories of quality and are used to assess barriers in terms of fish mobility. When assessing the connectivity in water bodies, the density of infrastructures that represent an obstacle to fish is calculated as the number of impassable barriers per WB length (Table 10). This indicator reflects impacts to

Table 8 Hydrological flow regime analysis (first step) combining water withdrawal degree (WW) and environmental flow compliance (EFC)

Water withdrawal degree (WW)	Environmental flow compliance (EFC)					
	Not assessed	High	Good	Moderate	Poor	Bad
High	High	High	Good	Good	Moderate	Moderate
Good	Good	Good	Good	Moderate	Moderate	Moderate
Moderate	Moderate	Good	Moderate	Moderate	Poor	Poor
Poor	Poor	Good	Moderate	Moderate	Poor	Bad
Bad	Bad	Good	Moderate	Poor	Bad	Bad
Not assessed	Not assessed	Not assessed	Not assessed	Not assessed	Poor	Bad

Table 9 Hydrological flow regime analysis (second step) combining quality class obtained in Table 8 and quality class from IHA

Combination between WW and EFC	Index of Hydrological Alteration (IHA)					
	Not assessed	High	Good	Moderate	Poor	Bad
High	High	High	Good	Moderate	Moderate	Bad
Good	Good	Good	Good	Moderate	Poor	Bad
Moderate	Moderate	Moderate	Moderate	Moderate	Poor	Bad
Poor	Moderate	Moderate	Poor	Poor	Poor	Bad
Bad	Bad	Bad	Bad	Bad	Bad	Bad
Not assessed	Not assessed	Not assessed	Not assessed	Not assessed	Poor	Bad

Table 10 River continuity assessment according to density of impassable fish barriers

Density of impassable fish barrier (barrier / km)	WB quality
<0.15	High
$0.16 < \text{Density} > 0.40$	Good
$0.41 < \text{Density} > 0.60$	Moderate
$0.61 < \text{Density} > 0.99$	Poor
>1.00	Bad

longitudinal connectivity as well as improvements when applying measures. In Catalonia, river continuity has been assessed taking into account transversal structures such as dams, weirs and gauging stations. Other elements such as bridges, sleepers and breakwaters have not been considered because of scarce information available and because their effects on river connectivity is potentially less significant.

3.3 Morphological Conditions and Quality of Riparian Zone

Morphological conditions and quality of riparian zone is calculated according to the HIDRI protocol combining the following elements: channelling stretch measurement (END), land use analysis on river banks and floodplain areas and the Riparian Quality Index (QBR).

3.3.1 Channelling Stretch Measurement (END)

Channel alteration on rivers is evaluated by means of a ratio between channelling stretch measurement and the total evaluated WB length. Channelling is considered as any artificial structure on the river margins mainly for flood control. These structures include walls, jetties, specks or "levees," gabions and any other engineering or bioengineering elements used for this purpose. If channelling affects both river banks (left and right margins), its length is computed twice. Depending on the protection structure, different weighting coefficients are applied (speck and other elements = 0.2; jetty and gabion breakwater = 0.5; wall = 0.8; wall and bed concreting = 1). The calculation is performed according to the following formula:

$$\mathrm{END} = \Sigma\,(\text{channelling length} \times \text{coefficient})\,/\,\text{WBlength}$$

The quality level is assigned as follows: high, less than 0.1; good, from 0.1 to 0.2; moderate, from 0.2 to 0.3; poor, from 0.3 to 0.4; and bad, higher than 0.4.

3.3.2 Land Use Analysis on River Banks and Floodplain Areas

The land use on the river banks and floodplain areas is estimated on a potential riparian buffer by means of geographic information systems (GIS) using habitat and land cover mapping. The riparian buffer width is estimated by applying a minimum width that depends on the cumulative basin area (CBA) of each WB: 10 m when CBA $\leq$ 20 km^2; 20 m when CBA is 21–100 km^2; 30 m when CBA is 100–200 km^2; 40 m when CBA is 200–1,000 km^2; and expert judgment when CBA $\geq$ 1,000 km^2. Afterward, this width is reviewed by photo-interpretation of present and past aerial photo-images as shown in Fig. 3. The oldest available aerial photo-image obtained in Catalonia is from 1956. This procedure ensures the inclusion of areas with fluvial physiognomy and with riparian vegetation structure and continuity, as well as with geomorphological patterns modelled by present and past water influence that helps the analysis. Additional information sources and criteria that contribute to the definition of the potential riparian buffer are also used in the Catalan rivers to assess the HYMO quality in floodplain and riparian areas: (i) information on geomorphology and mapped floodplains with return periods of 10 and 100 years, (ii) expert criteria in some water bodies and (iii) inclusion of relict patches and

Fig. 3 An example of photo-image obtained from 1956 with present riparian land uses shown above as coloured polygons used to assess the land use on river banks and floodplain areas in Catalan rivers

Table 11 Quality levels according to floodplain land use

	Quality level		
Land use (%)	High	Good	Less than good
Natural	≥85	60	<60
Agricultural	≤15	40[a]	>40[a]
Urban	0	5	>5

[a]Agricultural + Urban

habitats of fluvial influence in lower reaches where floodplains have more potential width and accordingly the potential riparian buffer increases.

Land uses are grouped into three categories – natural, agricultural and artificial – and afterward, the quality of land use is classified according to three classes (high, good and less than good) in each WB depending on the percentage of the existing land use categories in the riparian zone, as shown in Table 11. Natural areas are defined as dense and open forests, wetland vegetation, meadows and grassland, rocky areas, forest eroded soils, alluvial beaches, scrub and inland waters and also those areas occupied by the river channel. Agricultural uses include all traditional agricultural uses, as well as eucalyptus, poplars and deciduous species plantations, and urban uses mean urbanized and industrial areas and roads.

3.3.3 Riparian Quality Index (QBR)

The riparian zone quality is assessed through field works by applying the Riparian Quality Index (QBR) [13]. It establishes five quality levels (high, scored from 92 to 100; good, scored from 72 to 92; moderate, scored from 52 to 72; poor, scored from 27 to 52; and bad, scored <27). Since the QBR is applied on a relatively short river segment (100–200 m long), less than the total length of a WB, it is necessary to assess more than one sampling site in each WB to obtain a representative riparian quality result. By photo-interpretation or previous site visits, homogenous segments are selected in each WB based on riverbank and floodplain structure and land use physiognomy. Afterward, the QBR value of each site is extrapolated to its corresponding homogenous segment; therefore, the QBR value of an entire WB (QBR_{WB}) is determined by one or more QBR values. The procedure is as follows:

1. Compilation of QBR values from the Monitoring Surveillance Programme or from other monitoring networks or studies
2. Designation of homogeneous reaches in each WB based on land use and riparian zone structure using GIS and expertise criteria
3. Assignment of a unique QBR value for each WB as an average of the different QBR site values weighted by segment length with respect to the total WB length, according to the following formula:

$$QBR_{WB} = \Sigma\left(\times QBR_i(\text{length\_QBR}_i)\right) / \text{lenght\_WB}$$

where QBR_{WB} = integrated value for the whole WB; QBR_i = QBR score in a representative reach i; $Length\_QBR_i$ = reach length for each QBR_i; and Length_WB = total WB length.

If river banks and floodplain land uses are fairly homogeneous in a given WB, the QBR index will be assessed, at least, every 10 km. In the case that there are not enough QBR sampling sites, WB quality will be not assessed until information is gathered from more sampling sites.

The morphological conditions and the riparian quality are assessed first by combining results from land use assessment and the QBR index (Table 12), and the resulting quality is again combined with the channelling stretch measurement to obtain finally the morphological condition quality (Table 13).

Table 12 Riparian quality assessment combining land use and QBR index quality classes

	QBR index					
Land use	High	Good	Moderate	Poor	Bad	Not assessed
High	High	Good	Good	Moderate	Poor	Not assessed
Good	Good	Good	Moderate	Poor	Bad	Not assessed
Less than good	Moderate	Moderate	Moderate	Poor	Bad	Moderate

Table 13 Morphological quality assessment combining riparian quality and channelling stretch measurement quality classes

Channelling stretch measurement	Riparian quality				
	High	Good	Moderate	Poor	Bad
High	High	Good	Moderate	Moderate	Poor
Good	Good	Good	Moderate	Poor	Poor
Moderate	Moderate	Moderate	Moderate	Poor	Poor
Poor	Moderate	Moderate	Poor	Poor	Bad
Bad	Poor	Poor	Poor	Bad	Bad

3.4 HYMO Quality Assessment

The final HYMO quality assessment results from the combination of the three quality elements above mentioned according to the following criteria:

- Good: three elements with good or high quality
- Less than good (moderate or poor quality): one element with moderate or poor quality
- No assessment: any element without evaluation
- Bad: rest of situations

The results of HYMO quality assessment in the Catalan River Basin District (Table 14) highlight those problems that affect the ecological status due to physical habitat. On the other hand, HYMO quality assessment also improves the understanding of the results obtained from the mere analysis of the physicochemical and biological elements, as shown in Table 10. Regarding HYMO quality applied in Catalan rivers, only 14% of water bodies are considered with good or high quality. This is mainly due to the alteration of morphological conditions (54% of WB), reflecting problems derived from occupation of riverbanks or riverbank loss and significant alterations of river channel morphology (e.g. river channelling).

Changes in the hydrological regime because of water withdrawal, water diversion and flow regulation are the drivers of low quality in 17% of river WB. Although the percentage of WB with bad quality is low, the effects of this alteration on the ecological status are significant because stream flow directly affects biological communities. Therefore, low streamflow and flow regime alteration were identified as one of the major problems in the Catalan River Basin District to achieve good ecological status.

Finally, quality regarding river continuity is less than good in 13% of river WB because of the presence of more than 900 obstacles and barriers identified in Catalan rivers (considering dams, weirs and gauging stations) which heavily affect fish migration. Most of these barriers (over 800) are weirs (<15 m height), around 50 are large dams (>15 m height) and 97 are gauging stations. They are widely distributed throughout the Catalan River Basin District, but specially located in the

Table 14 Hydromorphological quality assessment in rivers of the Catalan River Basin District. The number of river WB with a given quality is shown as well as the percentage of them within the total river WB

	High	Good	Moderate	Poor	Bad	Without data
Hydromorphological quality (HYMO)	34 (14%)		96 (39%)		57 (23%)	61 (24%)

Llobregat basin (250 barriers), the Ter basin (165 barriers) and Besòs basin (151 barriers); these three basins contain around 66% of all Catalan River Basin District barriers. Results show the importance and usefulness of such HYMO diagnosis for the achievement of good ecological status.

4 Hydromorphological Quality Data and Ecological Status Assessment

HYMO analysis in rivers is a key issue to assess the complexity and heterogeneity of fluvial ecosystems [14], though physical habitat features are not as important as physicochemical parameters in assessing the ecological status of water bodies according to the WFD criteria. HYMO impacts and pressures are only considered important when they produce a deviation in biological communities, but not because of the effect they cause on physical habitats attributes per se. The effects of anthropogenic pressures on river hydrology, continuity and morphological conditions are poorly considered. It is worth to note that HYMO elements in the WFD are used in various steps: (i) to classify water bodies as natural, heavily modified or artificial; (ii) to identify reference sites and/or reference water bodies; (iii) to determine high ecological status; (iv) to identify human pressures; and (v) to design programmes of mitigation measures.

There is no scientific consensus for the establishment of reference hydromorphological conditions, though several authors have defined the geomorphic reference condition of a stream [50, 51]. Concepts such as guiding image and evolutionary trajectory [50] are largely accepted, while “pristine stream condition” is neither feasible nor worthwhile [51, 52]. Numerous debates in scientific literature show that the definition of reference conditions is not obvious and should be based on spatial aspects rather than on historical ones. In this respect, spatial aspects should comprise those set of river reaches considered to be as unmodified as possible by human pressures and not as static past conditions. The use of reference conditions based on statistical analyses of empirical data obtained from reference sites and pressure analyses might be not enough, and it requires an appropriate characterization of HYMO elements [53].

The HYMO alteration is considered to be the main factor that prevents the achievement of the environmental objectives of the WFD, but this does not apply for all European rivers. Impacts and pressures differ from permanent river systems to Mediterranean rivers, and also the application of mitigation measures differ from northern and central European water bodies to Mediterranean ones. In the latter aquatic systems, water availability is a determining factor for river biota; therefore, those measures that guarantee environmental flows are much more relevant than in-stream habitat improvements (connectivity, habitat complexity or substrate availability measures) [7]. Nevertheless, diversity and composition of biotic communities in streams strongly depend on multiple-scale factors, being land use the most important variable at catchment scale [54]. Low-flow conditions derive rapidly into water quality degradation as a confounding factor, and this situation must be considered when implementing WFD strategies. Insufficient river connectivity is considered as one of the main causes for the decline of many fish species in the Iberian inland waters. Improvement of river connectivity is needed to restore the natural population of fish and other aquatic organisms by enabling seasonal movements or migrations of aquatic biota and for them to reach feeding and reproduction grounds. Measures, e.g. barrier removal, are necessary, especially in those river sections which are crucial for the migration of native fish species. Finally, the quality of the riparian areas is strongly related with some important water ecosystem services, and thus, its conservation and, if necessary, its improvement are also relevant. Therefore, restoring HYMO conditions is an essential tool to achieve and preserve good or high ecological status. Management needs to be flexible by adopting spatial and temporal scales that research reveals to be critical for HYMO processes [44]. For instance, in a recent review on eco-hydrological methods (REFORM project [7]), measures for the improvement of HYMO features represented less than 15% of all river basin measures, while conceptual ones accounted for 70% of all total measures. In this way, programmes of measures do not have a proportionate relationship between problems (HYMO quality assessment) and solutions (measures). There is a need for information on cost-benefit analysis as well as on objective achievement in order to establish action scenarios.

5 Final Remarks and Conclusions

There is a lack of scientific and technical consensus on which HYMO methods to use and which river features to monitor, since fundamental questions on hydromorphological, chemical and biological characteristics and their interactions remain unanswered [42]. A more integrated view is needed to unveil the complexity of river processes and also to answer ecological questions such as those arising from the EC Habitats Directive. Fundamental restrictions remain on the ability to measure eco-hydromorphological patterns and processes in both time and space. There is a need to understand cause-effect relationships in eco-hydromorphology. Examples of unveiled responses stand in “point” HYMO modifications such as weirs or dredging and also in “diffuse” or distal HYMO modifications such as land use or climate change [42]. Mediterranean rivers are more sensitive to HYMO modifications, and thus, there is an urgent need to understand key HYMO elements and their relationship with biological communities.

The assessment of hydromorphological quality along with other screening or diagnosis tools (e.g. human pressures and impact analysis report: IMPRESS) are essential for targeting and/or prioritizing measures included in River Basin Management Plans. In those water bodies with good hydromorphological conditions, which are usually located in headwaters and/or tributaries with scarce presence of human activity, efforts should focus on preserving the good quality and preventing deterioration. In those water bodies with moderate to bad hydromorphological quality, restoration or improvement management measures are needed to achieve a good quality status and, thus, comply with environmental objectives. Although abundant information on WB is provided by monitoring programmes and IMPRESS analyses, there are inherent difficulties in transposing this diagnosis information into measures in River Basin Management Plans.

Monitoring programmes should include improved HYMO indicators and metrics in order to obtain a better fit with reality. Also, they should improve the diagnosis and assessment of water bodies in which there is not enough available information. Surveillance monitoring programmes are potentially the most important resource in this respect, because of their extensive coverage in space and time. A better understanding of eco-hydromorphology would emerge from refining and optimizing those existing monitoring programmes, by means of scientific rigour, and subsequently, valid conclusions would arise for river monitoring and management [42].

Methodological improvements both in terms of data collection (increasing accuracy and precision by means of remote sensing, drones or other innovative technological tools) and data analysis (using spatial and temporal analysis statistics) would allow a better understanding of temporal and spatial variability of aquatic ecosystems and would also allow filling some major gaps in Mediterranean aquatic ecology. In this sense, it is worth to note that physical habitat assessment methods generally require very detailed site-specific data collection, and thus, their application to a large number of WB might be difficult.

Most of those methodologies presented in this review focus on structures rather than processes. Moreover, these approaches do not consider the floodplain whose key features comprise past states and many habitats that are crucial for the ecological health of the river. Furthermore, some of the abovementioned approaches include indices that are applied to different riverine zones, i.e. IHF (stream bed) or QBR (riparian zone), and these need to be integrated in protocols to better understand the river complexity [4]. A part from this, the design of monitoring networks is also crucial for the detection of pressures and impacts as well as for the subsequent decision making according to monitoring findings. Different sampling site distribution, e.g. sites evenly distributed all over the watershed vs. sites concentrated on a few tributaries, could derive in different monitoring findings, and hence, different conclusions on water body status would arise.

It is also important to define which methodology must be developed and applied at each spatial scale (micro-mesohabitat, reach or catchment scale) because methodologies can vary accordingly, from simple to complex protocols, and the subsequent cost and effectiveness might be decisive. Nevertheless, the cost of monitoring is much lower than the cost of inappropriate decisions. There is a need to reinforce the hydromorphological role on status definition, by means of calibrated methodologies that integrate all those diverse HYMO features existing throughout all European river sceneries.

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Reviewing Biological Indices and Biomarkers Suitability to Analyze Human Impacts. Emergent Tools to Analyze Biological Status in Rivers

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Abstract The Catalan Water Agency has been testing and applying new methodologies and emergent tools over the last 20 years in order to enhance water quality monitoring in the Catalan River Basin District according to the EU Water Framework Directive (WFD) requirements. As a result the ecological quality of water bodies in Catalonia has been established and is currently monitored. Furthermore, bioremediation strategies are being implemented to improve the ecological quality of several water bodies. In relation to this the Catalan Water Agency is also devoted to assess and report to the EU that the applied remediation actions improved the quality of those water bodies. Most Mediterranean rivers suffer from water scarcity, and they are often located in densely populated areas. The combination of overpopulation with water scarcity translates into an overexploitation of water resources and consequently the deterioration of the ecological quality of rivers. Such deterioration in many places affects both the riparian habitat and water quality. Deterioration of water quality includes the reduction of water flow and the increase of pollution. Indeed in many occasions natural water flow is so low that effluents from wastewater treatment plants (WWTP) enter into the river with little dilution. Accordingly Mediterranean rivers are contaminated not only with persistent pollutants such as metals or persistent organic contaminants but also by pesticides, pharmaceuticals, personal care products, and other substances that, although they are not persistent, are continuously released into rivers from both diffuse sources and WWTP effluents. When this happens, the use of biomarkers and of laboratory or field toxicity assays offers the possibility to detect small changes in water quality, to identify detrimental stressors affecting aquatic biota, and to detect specific subtle

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effects such as those caused by endocrine disrupters. This chapter is structured in three main subchapters that address the suitability of biomarkers, in situ bioassays, and omic responses to assess effects of pollutants in river biota from Catalonian rivers.

Keywords Besós, Biological indices, Biomarkers, Ebro, Ecological status, Field bioassays, LLobregat, Omic

Contents

1 Introduction

Ecological assessment of water quality is fundamental to the management of surface waters and the protection of aquatic ecosystems. Biomonitoring of fresh waters are mostly based on measures of community structure, focussing on biological indexes estimated from riparian species. The Water Framework Directive (WFD) defines different Biological Quality Elements (BQEs) with regard of their composition and abundance. The declared BQEs are benthic algae (including macrophytes), phytoplankton, invertebrates, and fish. Zooplankton, a relevant component of the lacustrine food webs (see [1, 2]), is not considered. These BQE data can be matched with data relating to chemical pressures, the latter grouped into three types: those arising from general water chemistry problems (e.g., pH, oxygen), those arising from a lack or excess of nutrients (mainly N and P) and those arising from exposure to priority substances exceeding their Environmental Quality Standard (EQS) values, which are ultimately specified by the WFD, but by no means include all the potential hazardous compounds. In Tornés et al. [3], Fennessy et al. [4], Benejam et al. [5], and García-Berthou et al. [6], it is described with great detail past, present and future developments of BQEs for diatoms, macrophytes and fish. The information provided by the biological community can be summarized through several metrics, potentially useful as descriptors of multistress (see [2, 6–9]). These are specific metrics for biomass and others for community composition, which are widely used in management.

Nevertheless, BQEs are notoriously unspecific: they cannot respond to disturbances other than those they were developed to detect, making diagnosis of the actual impairment more difficult, and hence cannot be used for example for diagnostic purposes of specific pollutants. Identifying indicators of adverse change in ecological systems that can diagnose causal agents is a major challenge in environmental risk assessment [10]. Recently, the development of biological trait-based community indexes has allowed to diagnose effects of pesticides, salinity, and certain pollutants [11–14], but like the abovementioned BQEs, these indices can not respond to pressures other than those they were developed to detect, making diagnosis of the actual relevant pressures difficult. Furthermore, 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.

Community-based indexes are also affected by both habitat and water quality disturbances, thus making more difficult to identify particular stressors: viz., pollutants impairing water quality vs. habitat degradation. In relation to this, there are several studies showing that implementation of BQEs with measured of biological effects occurring at sub-individual levels may allow risk assessors to diagnose the cause of impairment and in many cases to detect subtle incipient detrimental effects on biota. In this regard, the integrated use of chemical analyses with effects of pollutants at the molecular level, in cells, tissues and organisms is a sound procedure for detecting impact of anthropogenic contaminants in freshwater systems and to identify cause–effect relationships. Moreover, since in real field situations aquatic organisms are currently being exposed to multiple chemical contaminants involving different toxicity mechanisms, each one contributing to a final overall adverse effect, the use of a large set of responses may allow us to identify the potential hazardous contaminants in the field [14, 15]. This information, then, can be used by Water Authorities to take actions to prevent further deterioration of ecological status.

2 Biomonitoring Tools: An Overview

The Catalan Water Agency has been testing and applying new methodologies and emergent tools over the last 20 years in order to enhance water quality monitoring in the Catalan River Basin District according to the EU Water Framework Directive (WFD) requirements. As a result, the ecological quality of water masses in Catalonia has been established and is currently monitored. Bioremediation strategies are also being implemented to improve the ecological quality of several water masses. Thus, the Catalan Water Agency is also devoted to assess and report to the EU that the applied remediation actions improved the quality of those water masses. In relation to this ACA has encourage and support the implementation of WFD biomonitoring methods with studies performed using biomarkers, lab and field toxicity assays. The advantages of such implementation are that they provide additional metrics and hence increase the likelihood to detect a biological change

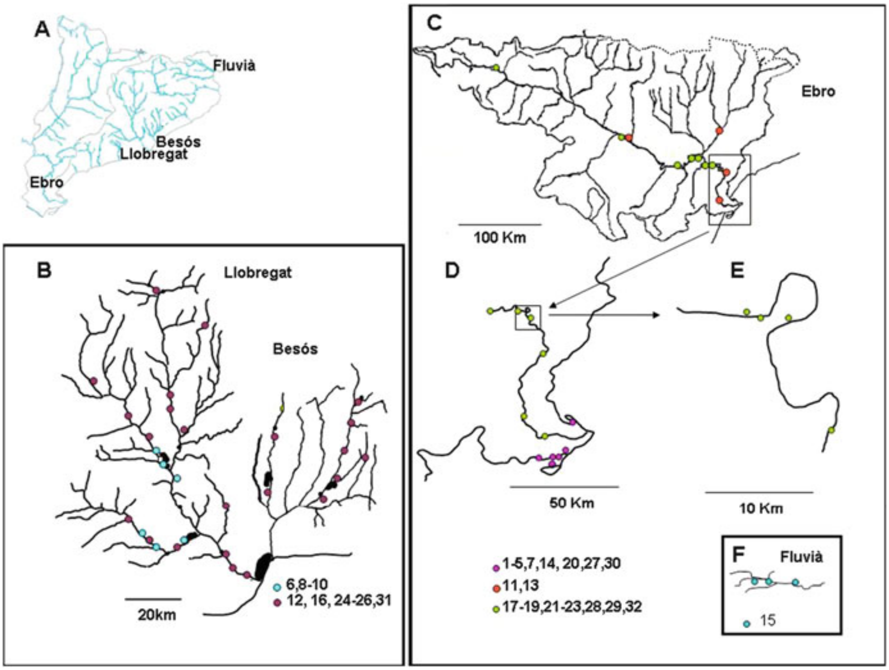

Fig. 1 Overview of the main biomonitoring studies conducted in Catalan Rivers in the last 20 years (**a**). Study sites within Llobregat–Besós (**b**), Ebro (**c**, **d**, **e**), and Fluvià (**f**) river basins are identified as *circles*. Studies are separated by species or/and research objective. In Llobregat and Besós studies were conducted to assess estrogenic effects mainly in fish (*blue*) or to diagnose physiological toxic effects in invertebrates (*purple*). In the Ebro river studies include assessment for endocrine disruption and other physiological alterations in fish along the river (*red*), effects of pesticides used for rice production in bivalves and crustacea (*pink*), and effects of sediment wastes from Flix (*green*). The *numbers* refer to the following studies: *1*. Escartín E, Porte C (1996) Environ Toxicol Chem 15:915–920; *2*. Escartín E, Porte C (1997) Environ Toxicol Chem 16:2090–2095; *3*. Porte C, Escartín E (1998) Comp Biochem Physiol 121C(1–3):333–338; *4*. Morcillo et al. (1999) Environ Toxicol Chem 18:1203–1208; *5*. Minier et al. (2000) Aquat Toxicol 50:167–176; *6*. Solé et al. (2000) Environ Sci Technol 34:5076–5083; *7*. Porte et al. (2001) Biomarkers 6:335–350; *8*. Fernandes et al. (2002). Environ Res 90:169–178; *9*. Solé et al. (2002) Aquat Toxicol 60:233–248; *10*. Solé et al. (2003) Comp Biochem Physiol 136C (2):145–156; *11*. Lavado et al. (2004) Toxicol Appl Pharmacol 196:247–257; *12*. Barata et al. (2005) Aquat Toxicol 74:3–19; *13*. Lavado et al. (2006) Environ Pollut 139:330–339; *14*. Barata et al. (2007) Environ Toxicol Chem 26(2):370–379; *15*. Damásio et al. (2007). Chemosphere 66:1206–1216; *16*. Damásio et al. (2008) Aquat Toxicol 87:310–320; *17*. Quirós et al. (2008) Environ Pollut 155:81–87; *18*. Navarro et al. (2009) Aquat Toxicol 93:150–157; *19*. Barata et al. (2010). Environ Pollut 158:704–710; *20*. Damásio et al. (2010) Ecotoxicology 19:1084–1094; *21*. Faria et al. (2010) Chemosphere 78:232–240; *22*. Faria et al. (2010) Chemosphere 81:1218–1226; *23*. Olivares et al. (2010) Sci Total Environ 408:5592–5599; *24*. Puértolas et al. (2010) Environ Res 110:556–564; *25*. Damásio et al. (2011) Chemosphere 85 (10):1548–1554; *26*. Damásio et al. (2011) Water Res 45:3599–3613; *27*. Ochoa et al. (2012) Sci Total Environ 437:209–218; *28*. Navarro et al. Sci Total Environ 454–455: 482–489; *29*. Navarro et al. (2013). Ecotoxicology 22:915–928; *30*. Ochoa et al. (2013) Mar Pollut Bull 66:135–142; *31*. Prat et al. (2013) Ecological Indicators 24:167–176; *32*. Faria et al. (2014) Aquat Toxicol 152:82–95

associated to a particular stressor. They also offer the possibility to detect subtle changes not detected, for example by BQEs, and hence they can refine WFD monitoring tools. In the next three sections we describe case studies on Catalan Rivers that used Biomakers (Sect. 3), field bioassays (Sect. 4), and omic responses (Sect. 5) to characterize risks of pollutants in Ebro, Llobregat, Besós, and Fluvià river basins. A graph summary of these studies is depicted in Fig. 1.

3 Biomarkers

There are many definitions of biomarkers. Here we select the definition of van der Oost (2003) that states that they are indicators of biological or biochemical effects after a certain toxicant exposure, which makes them theoretically useful as indicators of both exposure and effects.

Animals have been faced with a continual input of potentially toxic compounds. Central to the defense against such an enormous and diverse number of contaminants there is an impressive array of enzymes and biotransformation pathways involved in their detoxification and removal, but also those involved in the generation of molecular species, sometimes more toxic than the parent compound. So the potential sources of toxic molecular species, derived either directly or indirectly from the presence of contaminants, are the parent compound itself, reactive metabolites and free radical derivatives of the compound and enhanced production of toxic reactive oxygen species (ROS) [16]. There is also a broad array of biomarkers that are biological targets or specific by products of particular contaminants. For example inhibition of acethylcholinesterase activity is the target of organophosphorous and carbamate pesticides or the induction of vitellogenin in liver or plasma in fish males is a by product of exposure to estrogenic compounds. This means that studies conducted with biomarkers often use a broad array of biomarkers. In this section we describe case studies aimed to assess general stress and specific endocrine disruption effects on fish in Llobregat (Anoia tributaire), Fluvià, and Ebro rivers. Other studies that aimed to implement WFD with new metrics based on biomarkers developed in autochthonous benthic macroinvertebrate species and those that used transplanted macroinvertebrates to characterize and identify detrimental contaminants causing environmental hazards are also presented.

In the early 2000, enhanced plasmatic levels of vitellogenin in males of carps living near wastewater treatment plants discharging into a Llobregat river tributary (the Anoia river) [17–19] was described for the first time in Catalonia. These observations were coupled with elevated residues of estrogenic compounds in water such as nonylphenol and with intersex gonads (simultaneous development of male and female gonads, which is considered a female feature) and testicular atrophy. Few years later, Lavado et al. [20] also reported estrogenic effects in feral carps (*Cyprinus carpio*) collected in spring 2001 from five sites along the lower course of Ebro River (Spain). Several findings (low gonadosomatic index (GSI), plasmatic vitellogenin (VTG), depressed levels of testosterone, and histological alterations in gonads) detected in male carps downstream Zaragoza's sewage treatment plant (STP) strongly suggested that the concentration of sewage effluent in the area was a major causal factor leading to the detected estrogenic effects. Important alterations (viz. delayed maturation in females, indications of arrested spermatogenesis in males) were detected in carps from Flix, a heavily industrialized and polluted area. The previous studies provided the first evidence of the existence of significant alterations in the endocrine system of carps from the Llobregat and Ebro River basins. The combined use of biomarkers and chemical analyses has also been used to assess and identify pollutants causing detrimental effects. Fernandes et al. [21] sampled carps (*C carpio*) and red swamp crayfish (*Procambarus clarkii*) from two low-stream Mediterranean rivers (Anoia and Cardener) receiving extensive urban and industrial wastewater discharges. Tissue residues of selected pollutants (organochlorinated compounds) and biliary levels of polycyclic aromatic hydrocarbons (PAHs) were determined in conjunction with different biochemical responses (cytochrome P450, phase II enzymes) with the aim of investigating whether resident organisms were responsive to changes in water quality. Biota inhabiting those rivers were highly exposed to complex mixtures of polychlorobiphenyls and dichlorodiphenyl-trichloroethanes (up to 19 ng/g w.w.) and PAHs (up to 6,097 ng/g of hydroxylated PAHs in bile), the highest residues being observed in carps from Cardener River. These high levels of pollution translated into high activities of phase I detoxification enzymes such as that of 7-ethoxyresorufin *O*-deethylase (EROD) that in carps from Cardener ranged between 350 and 550 pmol/min/mg protein, whereas in carps from Anoia ranged between 90 and 250 pmol/min/mg protein. The highest EROD activity recorded was downstream of the sewage treatment plants in both rivers. Lavado et al. [22] collected carp (*C. carpio*) and barbels (*Barbus graellsii*) from five sites along the Ebro River. The study was designed to assess levels of persistent organic pollutants and metals bioaccumulated by fish, and some biochemical responses (cytochrome P450 system, phase II activities, and metallothioneins) against those pollutants. The highest levels of PCBs and DDTs were detected in carp from industrialized areas, which also showed high levels of mercury and cadmium in the liver, high levels of nonylphenol in bile, and high levels of EROD activity and of metallothionein proteins. Carps from the Ebro Delta, an agricultural area, had depressed acetylcholinesterase in muscle tissue. Years later, a remediation project was launched to study and characterize the toxicity of industrial wastes containing high concentrations of mercury, cadmium, and organochlorine residues dumped by a chlorine-alkali plant in a reservoir adjacent to the village of Flix (Catalonia, Spain), situated at the shore of the lower Ebro river. Effects of these contaminants to aquatic river biota were assessed in invertebrates [23, 24], fish [25, 26], and aquatic birds [27, 28]. Studies with invertebrates included zebra mussels, crayfish, Asiatic clams, and the native naiad species *Psilunio littoralis* [23, 24]. The results evidenced similar response patterns in bivalves and crayfish with increasing toxic stress levels from upper parts of the river towards the meander located immediately downstream from the most polluted site, close to the waste dumps. The aforementioned stress levels could be related with concentrations of mercury, cadmium, hexachlorobenzene, polychlorobiphenyls, and dichlorodiphenyltrichloroethanes that were 4- to 195-fold greater than local background levels. Using the common carp (*C. carpio*) the responses of EROD in liver, hepatosomatic index, condition factor, and the micronuclei index in peripheral blood showed maximal dioxin like effects in Ascó, few kilometers downstream the plant, where measured organochlorine residue levels in fish were the highest [26]. This combination of chemical, cellular, and physiological data allowed the precise assessment of the negative impact of the chlor-alkali plant on fish. Blood biomarkers of nestlings of the aquatic birds Purple Heron *Ardea purpurea* and the Little Egret *Egretta garzetta*, were also used to assess pollution effects of the industrial wastes of Flix on top predators that eat fish [27, 28]. Bird populations from Flix had the greatest levels of oxidative stress and of micronuclei in blood, which correlated with measured residues of mercury and of organochlorine compounds in feathers and eggs, respectively.

Characterization of the impacts of pesticides used in the rice fields of Ebro's Delta on biota living in the Delta or in its associated bays has been conducted for over 20 years. Pioneering studies were initiated by Cinta Porte in the common mussel and crayfish [17, 29–34], and years later were extended to *Daphnia magna*, Asiatic clams and oysters [35–38]. It is worth noting that bivalve species are quite resistant to the pesticides used in Ebro Delta probably since pesticides targets in these species (i.e., AChE) are quite insensitive to organophosphorous insecticides. The opposite happens with crustacean species that like arthropods are quite sensitive to anticholinergic pesticides like fenitrothion [38]. Nevertheless, in most studies detrimental effects were observed on the studied species. In crustaceans they were related to organophosphorous poisoning due to the inhibition of acetylcholinesterase, whereas in bivalves they were associated to major herbicides and fungicides usually related with increasing levels of oxidative stress.

Last but not least, in several studies biomarkers were used in combination with other metrics to solve specific environmental problems. Among them, we highlighted a study performed with the autochthonous fish species *Barbus meridionalis* in Fluvia River to assess the impacts of an oil spill [39]. Fourfold increase of EROD activity together with increased levels of fluorescent hydrocarbon compounds (FACs) in bile of barbs collected at the spilled site indicated exposure of inhabiting fish to the oil. Biological indices, mainly the diatom community IPS, showed slight significant effects between control and impacted sites, indicating that more tolerant taxa were favored because of the oil spillage. These results support the need to include biochemical responses measured in local species in monitoring programs aimed to diagnose specific pollution effects in stressed river ecosystems.

3.1 Biomarkers Developed in Autochthonous Benthic Macroinvertebrate Species

Several studies were devoted to develop biomarkers in local macroinvertebrate benthic species that dominate communities moderately and heavily impacted [40–42]. We select the caddisfly larvae *Hydropsyche exocellata* that is widely distributed along the Ebro, Llobregat, Besós, and other Mediterranean rivers due to its broad tolerance to salinity and pollutants, both major stressors that deteriorate water quality in Catalan Rivers. Biomarker responses in caddisfly larvae were used to detect sublethal effects and to get information on additional environmental factors that impaired benthic communities and could not be detected with BQEs. Up to ten different markers, belonging to distinct metabolic pathways, were developed and used to identify major contaminants affecting river biota in Llobregat and Besòs [40–42]. Results evidenced that salinity was one of the major stresses affecting macroinvertebrate assemblages, whereas antioxidant and metabolizing enzymes responded differently and were closely related to high and presumably toxic levels of measured organic pollutants. Those results indicated that the use of multiple -markers sensitive to water pollution may provide complementary information to diagnose environmental factors that are impairing macroinvertebrate communities. Indeed in other studies, we used the same experimental approach as above to assess undesired effects of remediation actions conducted by ACA on specific river basins. These included the use of reclaimed water to increase water flow and hence improve the ecological quality of rivers [37]. The discharge of the reclaimed water did not affect the composition and abundance of the dominant taxa, but the few intolerant species that were found upstream before the experiment disappeared downstream; consequently, most of the metrics indicating the level of biological impairment had slightly lower values after the introduction of the treated water, even though the ecological status was always poor. Nevertheless, significant and specific toxic effects on the collected *H. exocellata* larvae were observed using biomarkers. The effects included oxidative stress-related responses, such as decreased antioxidant enzyme activities and increased levels of lipid peroxidation. Therefore, indications of additional stress to the populations of the caddisfly *H. exocellata* were found using several biomarkers, which can indicate a potential further deterioration of the ecological status of the river. In polluted rivers, such as the Llobregat, structural indicators are unable to indicate further impairment and that biomarkers may be a useful tool to detect such changes. The combination of both kinds of indicators seems necessary for the establishment of the ecological status of a system, following the indications of the Water Framework Directive (WFD).

4 Field Bioassays

The Water Framework Directive (WFD) defines different Biological Quality Elements (BQEs) with regard of their composition and abundance. These BQE data can be matched with data relating to chemical pressures, the latter grouped into three types: those arising from general water chemistry problems (e.g., pH, oxygen), those arising from a lack or excess of nutrients (mainly N and P), those arising from exposure to priority and other substances discharged in significant quantities in the water body and exceeding their Environmental Quality Standard (EQS) values, which are ultimately specified by the WFD. The WFD also indicates that for those substances exceeding EQS it is recommendable to perform further studies using established ecotoxicological test. Toxicity testing in the lab suffers from the exposure problem since the lab exposures are too simple and not reflect the field environment. To solve this problem many scientists have developed field assays for example by caging lab populations in different sites and assessing effects on sub-individual, individual and even on community responses. The use of field bioassays allows minimizing the exposure problem since individuals are deployed in the field. It also may minimize the confounding effect of genetic adaptation arising for example when populations having different genetic background and hence adapted differently to the local conditions where they live, are used to monitor effects in the field. Due to these advantages field bioassays have been used also to study biomarker responses across a given stress gradient. Examples of the formed utility were used in the "mussel watch" monitoring program designed to study and monitor bioaccumulated pollutants and their effects. Here we are going to describe studies conducted with transplanted populations of *Daphnia*, macroinvertebrates, and freshwater mussels to monitor effects of pollutants in Llobregat, Besós, and the lower part of Ebro rivers.

D. magna acute and chronic toxicity assays are probably the most used test in aquatic toxicology. In the early 2000, the Scottish team from Stirling University leaded by Donald J Baird developed a *D. magna* field assay aimed to assess sublethal effects of pollutants on grazing rates using post-exposure feeding rates [43, 44]. The assay consisted in deploying individuals in specially designed cages into the field for 1 day after which animals were removed and their post-exposure feeding rates measured assessing algae clearance rates in the lab using clean medium and algae [43]. The *D. magna* post-exposure feeding field assay was robust and unaffected by other factors than pollutants like water temperature, alkalinity, pH, water flow rate, or suspended solids [43]. The previous characteristics make this assay more reliable than a previous one developed years before by Loraine Maltby from Shefield University in the amphipod species *Gammarus pulex* [45]. The amphipod assay measured in situ grazing rates on leafs and need it match more time of exposure, which make the assay affected by water temperature and other physical chemical confounding factors. Nevertheless, now there are many field assays out there that measure individual responses of several species of algae, invertebrates, and vertebrates linked with fitness such as grazing rates, growth, mortality, and reproduction. The team of Isabel Muñóz from Barcelona University has developed one with the local aquatic snail species *Physella acuta* that it can measure effects of pollutants on fecundity [46]. This bioassay has been successfully applied in the Llobregat and Ebro Rivers to evaluate impacts of potential estrogenic compounds on aquatic snails. In our lab, we improved the *D. magna* field assay combining post-exposure feeding measures with several biomarkers and gene transcription responses. The improved assay was used for the first time in Ebro's Delta (North East Spain) [38]. The aim of this investigation was to evaluate toxicity effects of pesticides in aquatic invertebrates by using in situ bioassays with the local species of *D. magna*. Investigations were carried out during the main growing season of rice (from May to August). Measures of energy consumption (i.e., algal grazing) and of specific biochemical responses (biomarkers) were conducted in individuals transplanted in four stations that included a clean site upstream of the affected area and the three main channels that collect and drainage the water from the rice fields into the sea. Seventeen pesticides were analyzed in water by online solid phase extraction-liquid chromatography–tandem mass spectrometry (LC-MS/MS). The results obtained indicated high levels of pesticides in water with peak values of 487 μg/L for bentazone, 8 μg/L for MCPA, 5 μg/L for propanil, 0.8 μg/L for molinate, and 0.7 μg/L for fenitrothion. Measured biological responses denoted severe effects on grazing rates and a strong inhibition of cholinesterases and carboxylesterases, which are specific biomarkers of organophosphorous and carbamate pesticides, and altered patterns of the antioxidant enzyme catalase and the phase II metabolizing enzyme glutathione-*S*-transferase. Correlation analysis with pesticide residue levels converted to toxic units relative to its acute 48 h median lethal concentration effects (LC50) of *D. magna* indicated significant and negative coefficients between the dominant pesticide residues and the observed biological response, thus denoting a clear cause–effect relationship. A second study aimed to assess the feasibility of using the post-exposure *D. magna* feeding assay in combination of BQE metrics to identify environmental factors affecting aquatic invertebrate communities [47]. Investigations were carried out in two heavily industrialized and urbanized river basins from the NE of Spain (Llobregat and Besós). Measures of energy consumption (i.e., algal grazing), and of specific biochemical responses (biomarkers) were conducted on individuals transplanted upstream and downstream from effluent discharges of sewage treatment plants. In both rivers there was a clear deterioration of the ecological water quality parameters and of benthic community BQEs towards downstream reaches. In all but one of the 19 locations studied, transplanted organisms were affected in at least one of the five measured responses. In three of them, significant effects were detected in most of the traits considered. Principal Component and Partial Least Square Projections to Latent Structures regression analyses indicated that the measured responses in *D. magna* in situ bioassays and those of macroinvertebrate assemblages were affected by distinct environmental factors. From up to 20 environmental variables considered, seven of them including habitat degradation, suspended solids, nitrogenous and conductivity related parameters affected macroinvertebrate assemblages. On the other hand, levels of organophosphorous compounds and polycyclic aromatic hydrocarbons were high enough to trigger the responses of *D. magna* in situ bioassays. These results emphasized the importance of combining biological indices with biomarkers and more generalized and ecologically relevant (grazing) in situ responses to identify ecological effects of effluent discharges from sewage treatment plants in surface waters. In two additional studies, the in situ *D. magna* post-exposure feeding assay in combination with other matrix were used to address specific problems of The Catalan Water Agency. Puertolas et al. [48] evaluated side-effects of glyphosate mediated control of giant reed (*Arundo donax*) on the structure and function of a nearby Mediterranean river ecosystem. One of the main causes of river degradation is the presence of invasive alien species which pose a significant threat to the ecological integrity of river ecosystems. Alien species are often cited as the second most pressing threat (after direct habitat destruction) to global biodiversity. Giant reed (*Arundo* donax) is an invasive plant for riparian habitats and can be considered a primer riparian management problem. As river restoration has become a priority for water authorities and river managers in many countries, several methods for controlling this plant have been attempted and among them chemical control with nonspecific herbicides. Glyphosate is a broad-spectrum systemic herbicide that has been used to control a wide range of weeds; during the past four decades it has also been applied to control exotic or invasive species. Many commercial herbicides have been formulated using glyphosate (isopropyl amine salt) as active ingredient. On behalf of a river restoration project to control the giant reed, glyphosate was applied in the riparian vegetation across a restricted area in the mid section of the Llobregat river basin. The aim of this study was to evaluate the effect 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*) on the structure and function of a nearby river ecosystem. Specifically, we assessed glyphosate environmental fate in the surrounding water and its effects on transplanted *D. magna*, field-collected caddisfly (*Hydropsyche exocellata*), and benthic macroinvertebrate structure assemblages. Investigations were conducted in the industrialized and urbanized Mediterranean river Llobregat (NE Spain) before and after a terrestrial spray of glyphosate. 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. 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, 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.

Caged organisms can also be used to study detrimental effects of particular pollutants in the field in certain species that are not very abundant. Caged organisms also minimize the problem of adaptation when interpreting phenotype responses [49]. In several studies we have used caged organisms to study the effects of mercury release by the chlor-alkali industry of Flix on invasive and autochthonous freshwater mussels [24], the effects of pesticides used in Delta del Ebro for rice production on bivalve populations and the impact of pharmaceuticals [35], metals, and other contaminants in local macroinvertebrate species along the Llobregat river [41]. The results of these studies have been already reported in Sect. 3.1.

5 Omic Technologies

The analysis of changes in gene expression represents a potentially powerful tool to characterize immediate cell responses to stressors, constitutes an early warning of the effect of contaminants, and represents a useful complement to existing monitoring methods to study the effects of toxicants at the biochemical level [50–52]. Among the techniques for specific RNA quantification, quantitative real-time PCR, or qRT-PCR, has become one of the most sensitive tools in Molecular Biology, allowing detection of truly minimal amounts of RNA molecules by amplification of specific sequences. With appropriate extraction and analytical methodologies, as few as 10–100 RNA molecules can be detected and, with some limitations, quantified. The high reproducibility and sensitivity of qRT-PCR allow both the application to small individuals (small animals, for example) and the use of dispensable parts of the body (scales, blood, blubber), avoiding the killing of larger animals [53–55]. Being amenable to high-throughput screenings, qRT-PCR allows analyzing many individuals and, therefore, the study of ecological impacts at the population, rather than at the individual, level.

The only true limitation for the application of gene expression biomarkers in biological monitoring is the knowledge of appropriate DNA sequences: any gene can be analyzed in any species provided its sequence is known. This is particularly important in analyzing natural populations, whose dominant species are, more often than not, poorly described in terms of gene sequences [52]. Another major point is the choice of the tissue to be sampled. Liver is the preferred organ for fish species, although it requires dissection of the animal and, in some cases, it may not reflect the actual response of the species to some environmental injuries. For example, in a survey of the physiological responses of carps (*C. carpio*) from the Low Ebro River, we found that expression of kidney metallothionenins (MT-I and MT-II) reflected the levels of mercury present in the specimen, not only in kidney but also in liver and muscle, whereas expression of the same genes in the liver seemed unaffected by mercury poisoning (Fig. 2). In fact, our data suggest that CYP1A expression, the genetic counterpart of EROD activity, did reflect major physiological alterations linked to pollution (hepatosomatic index, condition factor), related in this particular case to organic pollutants, whereas mercury seemed relatively less toxic for the animals, but affecting specifically the metabolism of kidneys (Fig. 1a [56, 57]). This kind of analysis, in which effects on different tissues are compared and related to external stressors, is far more doable using qRT-PCR techniques than with standard biochemical methods.

Microarray analysis allows a simultaneous quantification of a large number of genes, helping to determine the phenotype of a given individual in a particular environment as well as the identification of altered metabolic paths, which may allow to identify potential detrimental stressors including pollutants [52, 58]. However, microarray analysis requires a substantial knowledge of the species genome and Molecular Biology, two aspects that are particularly lacking for aquatic organisms of ecological relevance [52, 59]. In spite of this general requirement,

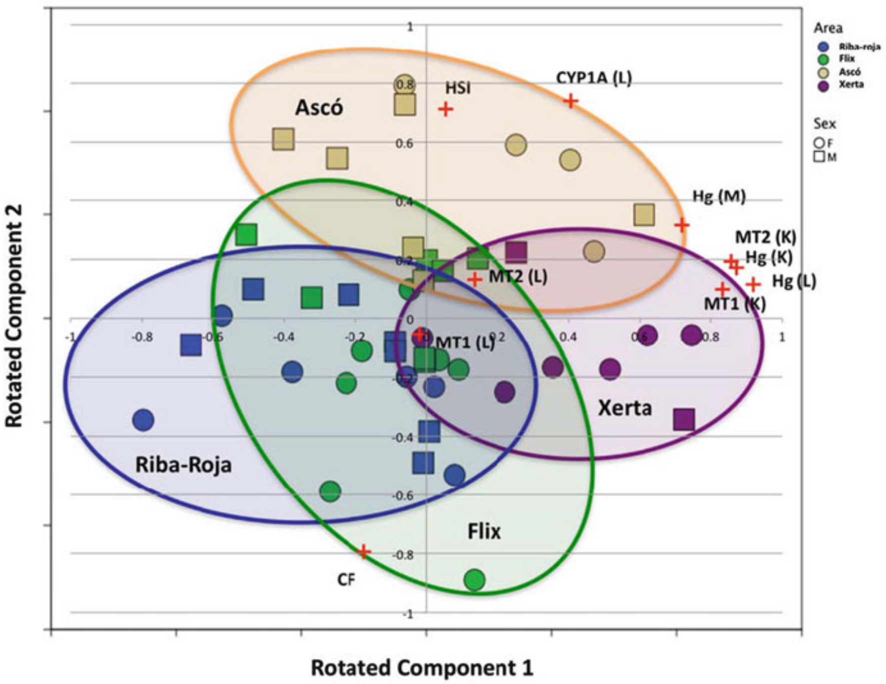

Fig. 2 PCA analysis of qRT-PCR results from different carp populations in the Ebro River. *Blue*, *green*, *brown*, and *magenta* symbols refer to samples from Riba-roja, Flix, Ascó, and Xerta sampling points, *circles* correspond to females and *squares* to males. The approximate distribution of each set of samples (score values) is limited by *ovals of the same color*. *Red crosses* indicate loadings for the different parameters included in the analysis: Mercury quantitation (Hg), Condition factor (CF), Hepatosomatic Index (HSI), and gene expression data from CYP1A and the metallothioneins MT1 and MT2. "L," "M," and "K" indicate data from liver, muscle, and kidney, respectively. Score values are represented scaled to fit the −1/1 interval

microarray analyses can provide very useful information about the physiology and the responses to pollution of poorly know species (at the genome level), provided a reliable and relatively large set of expressed RNA sequences are available. In an analysis of the zebra mussel (*Dreissena polymorpha*)2 populations established in the Ebro River, we designed a microarray with some 3,500 expressed sequence tags (ESTs) from different *Dreissena* species [60, 61]. Using RNA from zebra mussel samples from different points in the low Ebro River, we found a continuous gradation in the expression of at least two sets of (putative) genes. Genes related to proliferation, respiration, and cell signalling (function that we link to the normal physiology of the cells) were more expressed at the upstream sampling areas, with low pollution impact, whereas stress- and structural-related (including ribosomes) genes were expressed at the impacted, downstream populations (Clusters B and A in Fig. 3a, respectively [61]). Correlation analyses showed that expression of genes from Cluster B correlated with known markers of healthy status of zebra mussel,

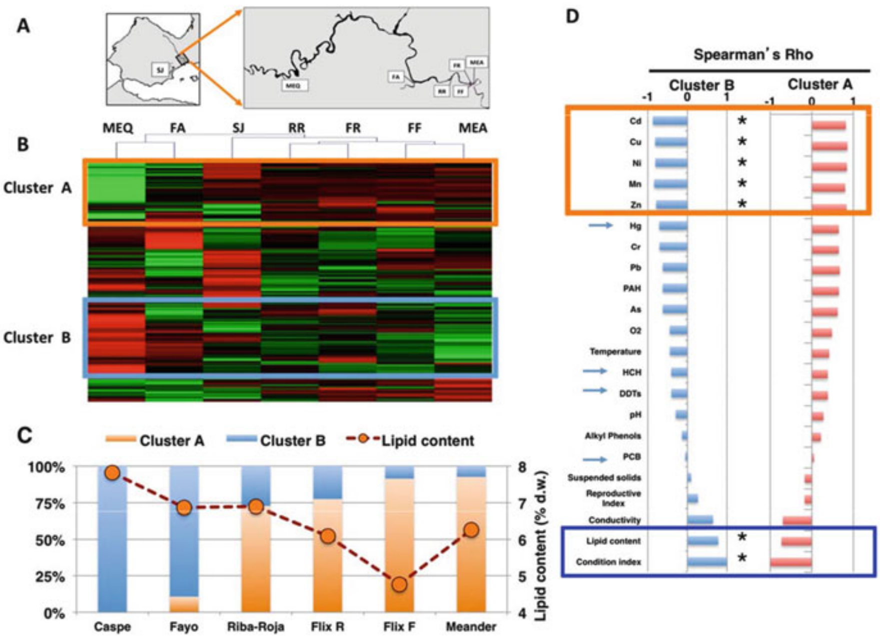

Fig. 3 Overview of the transcriptomic analysis of the Ebro River zebra mussel populations. (**a**) Sampling sites. Ebro River sites: MEQ: Mequinenza reservoir; FA, Fayón, Riba-Roja Reservoir; RR, Riba-Roja town, Flix reservoir; FR, site at the opposite site of the chlor-alkali industry, Flix reservoir; FF, site besides the chlor-alkali industry, Flix reservoir; MEA, Flix meander, downstream the Flix reservoir. Site SJ corresponds to the Sitjar reservoir, at the Mijares River. (**b**) Hierarchical clustering of the microarray data. Only the 1,000 features showing the highest variability among samples are included. Clusters A and B are indicated by *orange* and *blue squares*, respectively. (**c**) Relative expression values of genes from clusters A and B for the different zebra mussel populations (color codes as in **b**). *Dots* represent average lipid content values in % of dry weight. (**d**) Correlation analysis (Spearman's Rho) for cluster A and B genes with different chemical and physiological parameters. *Asterisks* indicate significant correlations ($p < 0.05$), *orange and blue boxes* indicate those parameters directly correlated to cluster A and B, respectively. *Blue arrows* point to the major pollutants released by the Flix factory

like the condition index or lipid contents (Fig. 3c, d), confirming the relationship of these genes with the normal cell physiology. Conversely, and unlike the results from carps, mercury and organic pollutants seemed not to play any significant role on the physiological status, as their levels did not show any correlation with either cluster (Fig. 3d). Rather, expression of stress genes did correlate with the concentrations of several heavy metals, like Cd, Cu, Ni, Mn, and Zn. Therefore, our results indicate that zebra mussels are not particularly sensitive to the major organic pollutants released by the Flix factory, being the concentration of heavy metals the major stressors for these populations in the lower part of the Ebro River.

6 Conclusions

A great effort to enhance quality status in the Catalan River basins has been carried out through sewage plant construction and habitat restoration by the Catalan Water Agency and local institutions. This 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 been analyzing the quality status and biota in the Catalan River basins since long time ago. This is true for the Llobregat river, where substantial work has been performed [62], and the Ebro river although for the latter river management actions depend on the Hydrographic Confederation of Ebro. The quality and abundance of such information has been a key element to fulfill the challenge of improving the ecological status of those rivers, and to establish a suitable monitoring program. Mediterranean rivers from Catalonia suffer a considerable ecological impact basically due to human pressures throughout their river basins. The most important anthropogenic impacts included salt mine activities, hydropower water diversion, and flow regime alteration by dams in headwaters and mid basins, together with urban and industrial sewage discharges. Some programs of measures have been progressively applied along time in order to mitigate such impacts, which include the build of sewage treatment plants 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 river basins. Such amelioration has allowed restoring some biological communities but not fish or native freshwater bivalves, aquatic birds, and mammals. Some anthropogenic pressures are still remaining. The high amount of weirs and hydropower water diversion along the 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 basins, 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 river basins cause the detection of some priority substances and emergent pollutants (e.g., endocrine disruptors, heavy metals, pesticides, brominated flame retardants, drugs, pharmaceuticals), which all together increase the ecological threats. Biomonitoring studies carried out in some of the Catalan river basins provide insights that biomarkers and field bioassays may also inform us of the actual ecological status when used together with community indices. Although biomarkers play a great role in ecotoxicology and environmental risk assessment, they are sometimes difficult to interpret [63]. It is problematic to determine whether a single biomarker response is an indicator of impairment or is a part of the homeostatic response, indicating that an organism is successfully dealing with the exposure [63]. However, the use of large set of biomarkers representing several metabolic paths overcomes problems of interpretation and as shown in this study allows characterizing true 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 lab sensitive species such as those of *D. magna* may also allow standardizing field assays. Such field assays are experimentally robust and reliable.

In the future, in addition to community indices, biomarkers, although they are not incorporated in the WFD, should be considered as tools for implementation of the WFD. 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 value between moderate and good.

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Analysis of EU Legislated Compounds for Assessing Chemical Status: Main Challenges and Inconsistencies

Josep Caixach and Arancha Bartolomé

Abstract The list of priority substances from the EU Water Framework Directive (WFD) (2000/60/EC) was recently revised (Directive 2013/39/EU). A total of 12 new priority substances were added, and some EQS values were also modified. For different reasons (toxicity, uses, and environmental fate), the proposed EQS values are extremely low, and it is the need to reach excessively low quantification limits. This chapter considers challenges and limitations of analytical methodologies and, according to literature and the *state of the art* of our laboratory, explains the difficulties for routine laboratories to achieve some EQS values.

Keywords Analytical methodologies, Chemical status, EQS, LOQs, Priority substances, WFD

Contents

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Abbreviations

AA	Annual average
DEHP	Di(2-ethylhexyl)-phthalate
EQS	Environmental quality standard
GC-Q	Gas chromatography-quadrupole
HPLC	High performance liquid chromatography
HRGC	High-resolution gas chromatography
HRMS	High-resolution mass spectrometry
IDMS	Isotope dilution mass spectrometry
JRC	Joint Research Center
LOQ	Limit of quantification
MAC	Maximum allowable concentration
MS/MS	Tandem mass spectrometry
NP	Nonylphenol
OP	Octylphenol
PAH	Polycyclic aromatic hydrocarbons
PBDE	Polybrominated diphenyl ethers
PS	Priority substances
QA	Quality assurance
QC	Quality control
SCCPs	Short-chain chlorinated paraffins
US EPA	United States Environmental Protection Agency
WFD	Water Framework Directive (2000/60/EC)

1 Introduction

The Water Framework Directive (WFD) of the European Commission (2000/60/EC) [1] describes the monitoring of priority substances in surface water of the European Union. The daughter directive 2008/105/EC [2] defined the environmental quality standards (EQS) for priority substances (PS) in water, with the aim to protect the aquatic environment. The PS has been defined as substances presenting a significant risk to or via aquatic environment at EU level. In order to assess risk, both hazard and exposure need to be considered. The list of PS was recently revised (Directive 2013/39/EU) [3], a total of12 new priority substances were added, and some EQS values were also modified. Values are defined as annual averages (AA-EQS) and maximum allowable concentrations (MAC-EQS). Moreover, some additional biota values were included.

For various reasons, such as toxicity, uses, and environmental fate, in some cases or substances, the proposed EQS values are extremely low. In that case, along with the QA/QC parameters of the analytic methods (2009/90/EC) [4], there is the need to reach “excessively” low quantification limits (LOQs). Additionally, remember that compliance monitoring for the PS in the WFD requires the achievement of a

LOQ equal or below a value of 30% of the relevant EQS. The achieved method quantification limits are therefore 0.3xEQS.

The norm does not specify if there are AA or MAC or whether there is a family or individual substance. This is even more critical in coastal waters where the existing precautionary principle requires lower values of EQS. This issue has caused an interesting analytical discussion that involves the overcoming of some analytical challenges – state of art – and it results in some inconsistencies worth mentioning.

This chapter discusses what substances are feasible for routine methods (official control laboratories), which substances require more sophisticated analytical methods, and which ones – despite all the strategies of the sampling and instrumental – remain above the proposed EQS. Therefore, some questions arise in this situation: What should the government do? How to define the chemical status of the affected water bodies? Or why are EQS values proposed when the analytical community or the same technical committees of the EU know that these are difficult to achieve?

We are not going to discuss neither the benefits nor the intentions. We will focus on the analytical results or analytical methodologies that will give valid results or robustness to the analytical determinations required in achieving the EQS and allowing the intercomparison.

2 Challenges and Limitations of Analytical Methodology

The application of WFD raises a number of analytical challenges that can be summarized as:

- Work to have the best available methods to obtain the lowest possible LOQs according to EQS.
- Apply the best laboratory practices for a reliable/consistent result (QA/QC).
- Validate methods and results participating in interlaboratory exercises aiming at monitoring data of sufficient quality to ensure harmonization or intercomparison.
- With the purpose of risk assessment for future identification of PS, in particular as regards emerging pollutants, the Directive has introduced what they call watch list [3]. The mechanism will ensure the targeted collection of monitoring data on the concentration of substances in the aquatic environment. The proposed list of substances to be monitored has been subject of numerous meetings and discussions within the Commission. These substances will be monitored in a limited number of representative stations across Europe to gain high-quality information to assess the potential risk posed of emerging pollutants and in consequence set reasonable EQS and help to make a validation of analytical methods used in monitoring and provide suitable analytical protocols with the

aim of shortening the necessary standardization process. The monitoring will be in water, sediments, or biota.

These issues come from some inconsistencies in the implementation of WFD that should be reconsidered:

- The mix of protocols and criteria (toxicological/use/monitoring) and various commissions originates unrealistic EQS and analytically intractable LOQs, resulting in lack of robustness of the method.
- Concerning the determination of LOQ, the Directive 2009/90/EC [4] does not specify from which EQS (AA or MAC) should be done.
- What is the LOQ for each compound in the case where EQS is defined for a sum of substances? Do you have to divide 30% of EQS between the numbers of congeners?
- Monitoring data from literature for the inclusion in the proposals, it is desirable that all are scrutinized according to the same criteria or the comparison with the performance and robustness/reliability of the analytical methods used.
- There is the need of improvements in the sampling and analytical methodological and instrumental capabilities to allow widespread adequate measurements.
- Despite sediment as an important compartment for its ability to bioaccumulate, and the existence of guidance to chemical monitoring [5], nowadays there is not EQS defined for this matrix yet. Members states had the order to set up commissions to work on deriving EQS for sediment [6]; there has not been consensus on this issue. According to Directive 2013/39/UE [3], member states could monitor PS on this matrix applying the relevant EQS. In any case, member states shall take measures aimed at ensuring that concentrations do not significantly increase.

3 Discussion

3.1 *Limitations of Analytical Methods and Harmonization Exercises*

Intercomparison exercises are the most practical and operative ones that give validity to the analytical methods applied. It is a good tool to highlight analytical problems and harmonize analytical methods. In this context, the European Commission, through the Joint Research Center (JRC), organized the Chemical Monitoring Activity Exercises (CMA on-site). The main objective was focused on assessing the limitations of analytical methods for some groups of PS. Three exercises were organized: the first one took place on the River Po in October 2006 (CMA on-site 1), the second one on River Danube at Budapest in September 2008 (CMA on-site 2), and the last one in October 2010 on River Meuse at Eijsden (the Netherlands) (CMA on-site 3). Different laboratories that participated were

Fig. 1 Method performance for PBDEs WFD monitoring**.** Number of laboratories ready (*green*) or not ready (*red*) for the sensitivity requirements of 30% EQS (1/6×30% of 0.5 ng/L sum of BDE congener numbers 28, 47, 99, 100, 153, and 154 equals LOQs of 0.025 ng/L for each single congener) as specified in the proposal for the Commission Directive on technical specifications for chemical analysis and monitoring of water status for WFD chemical monitoring [8]

chosen as representative of member states. The results and conclusions of the three exercises were published [7].

In our opinion, the results of CMA exercises 2 and 3 give an example of the difficulties of harmonizing analytical methods and comparing the results obtained by different laboratories in "conventional" families. The findings are conclusive in this respect.

Figures 1, 2, and 3 show some of the results of CMA on-site 2, in which samples from the River Danube located downstream Budapest city were analyzed with a joint exercise of sampling and a subsequent analysis with methodologies that each laboratory had readied.

The conclusions of the CMA on-site 2 studies are especially relevant [8]:

- Environmental concentrations of PAH, PBDE, and NP/OP can be analyzed in surface waters at concentrations taking into account the set European Environmental Quality Standards values and the proposed performance criteria.
- Among the included analytic groups, PBDE appear to be a major challenge monitoring at sub-ng/L level in water samples.
- Very much differing sampling and analytical methodologies are still in use within Member States.
- Not all among the participating laboratories were able to deliver results at the required concentration levels.
- No proficiency testing scheme or other external quality control possibility, taking into account the problematic of real environmental samples, is available at present for these analyses.

Fig. 2 Method performance for nonyl-/octylphenol WFD monitoring**.** Number of laboratories that are ready (*green*) or not ready (*red*) for the sensitivity requirements of 30% EQS as specified in the proposal for the Commission Directive on technical specifications for chemical analysis and monitoring of water status for WFD chemical monitoring [8]

Fig. 3 Method performance for PAHs WFD monitoring**.** Number of laboratories ready (*green*) or not ready (*red*) for the sensitivity requirements of 30% EQS as specified in the proposal for the Commission Directive on technical specifications for chemical analysis and monitoring of water status for WFD chemical monitoring [8]

- In vicinity to the proposed EQS concentration levels, high data quality is of importance for compliance checking.
- Blank values in analytical procedures are of crucial importance, as analytical problems can lead also to an overestimation of pollutant content and consequently even noncompliance.
- The occurring variability of contaminants in surface waters is of utmost importance for the selection of the monitoring strategies and needs therefore to be studied.

The 5-year period (2006–2010) on CMA on-site exercises provides a picture of the development of harmonization level of selected monitoring methodologies in EU Member States.

The more relevant conclusions [7] add to the above would be:

- It was evident that not all participating laboratories were able to deliver results at the required concentration levels. Furthermore, we obtained in some cases very high data variability, which represents a problem in compliance checking.
- The reduction of the variability among laboratories should be the most important goal to be achieved for the harmonization of WFD monitoring around Europe.
- Investigating gaps in analytical performance can help to identify needs for further development strategies and methodologies. Examples of such issues are the analysis of whole water and the variability of concentrations in surface water.
- While the requirements can change with the legislative context (e.g., revision of the EQS Directive), there is a clear need to continue harmonization at different organizational levels.

3.2 Need of Most Advanced Instrumentation and Methodologies

According to Directive 2009/90/EC [4] concerning technical specifications for chemical analysis, the need of most advanced instrumentation is obvious. MS/MS (HRGC or HPLC) methods are regularly used today, we do not see why we should renounce to the most advanced methods based on criteria that is not a routine method, economic high cost, etc. Methods like HRGC-HRMS and HPLC-HRMS are common in many methodologies.

US EPA is innovative using best available analytical methodologies as IDMS and HRMS (methods 1613 [9], 1614 [10], or 1668 [11]) or IDMS and MS/MS (method 1664 [12]). However, currently, the methods MS/MS are coming with a good state of the art and sensibilities almost as good as of HRMS. Therefore, there is no serious argument for not allowing the use of it to reach the fulfillment of lower EQS. Furthermore, there are prescreening strategies available.

New advances have been introduced in the field of instrumentation, HRMS Orbitrap analyzer, and recently GC-Q Orbitrap. This instrumentation is going to allow an important advance toward getting better quantification limits. It is noteworthy that the HRMS gives robustness to analytical methods minimizing the effect of the matrix and also the potential inaccuracies in quantification in the analysis by liquid chromatography tandem to mass spectrometry. HRMS is a good approach for combining the qualitative and quantitative analysis together. Remember that misuse of MS/MS has led to many false-positives or questionable results that have remained described at the literature. In our opinion, many environmental data are questionable for this fact. We would like to highlight again the importance of intercomparison exercises.

Another point to keep in mind is that the use of this instrumentation is not easy or a routine in many cases. Hence, it is very important to have trained personnel.

Nevertheless, with the use of advanced instrumentation, to date, there are many analytical problems with some substances. The works of Vorkamp [13] and Loos [14] give us the exact extent of the limitations of the analytical methods regarding the compliance of the proposed EQS in Directive2013/39/EC [3]. It will be developed later.

Among others technical requirements, LOQ must be equal or below a value of 30% of the relevant EQS [4]. This is one of the goals of the analytical methods. The LOQs given are linked to a specific methodology and instrumentation and current approach of water volumes [14], and an adequate state of the art could improve it. However, LOQs are not constant values and can change over time. They are dependent on several parameters and hence have to be verified regularly [14].

The blanks of laboratory/method are one of these parameters. The values obtained show the "reality" of LOQs and may invalidate all the effort improving the sensitivity of instrumental methods. One of the PS that present many problems with blanks are PBDEs that are widespread in the laboratory environment. As an example, Table 1 shows levels of PBDEs obtained in pristine waters from a high mountain lake used as blanks of method to calculate LOQs. You have to realize that the concentration obtained for the sum of legislated PBDEs is in the same level of required LOQ for coastal waters (0.06 ng/L) under Directive 2008/105/EC [2]. In the case of PBDEs, the EQS has changed, but we want to highlight this problem that occurs with other PS as DEHP (LOQ required 0.39 μg/L) or naphthalene (0.6 μg/L).

In the improvement of the analytical methods, the use of isotope dilution mass spectrometry (IDMS) is a very good tool. IDMS consists in the use of isotopically labeled analogues as internal standards considering that the natural sample contains negligible amounts of them. The isotopic analogue is added to the sample at the very beginning of the analytical method; it enables exact compensation to be made for errors at all stages of the analysis [15]. IDMS gives accurate, robust, and reliable results [16, 17]. However, the use of IDMS has a number of advantages and disadvantages, which the user should consider [15]. Therefore, the method

Table 1 Levels of PBDEs in pristine waters used as blanks of laboratory. *Source*: Laboratory of Mass Spectrometry-Organic Pollutants

Sample	Pristine groundwater (ng/L)	Surface water[a] (ng/L)	Deep water[a] (ng/L)
Compound			
BDE#28	0.002	0.002	0.002
BDE#47	0.011	0.023	0.018
BDE#99	0.008	0.014	0.012
BDE#100	0.003	0.005	0.003
BDE#153	n.d	n.d	n.d
BDE#154	n.d	n.d	n.d
BDE#183	n.d	n.d	n.d
BDE#197	n.d	n.d	n.d
BDE#209	n.d	0.037	n.d
ΣLegislated BDEs	0.024	0.044	0.035

n.d: not detected
[a]Water from high mountain lake

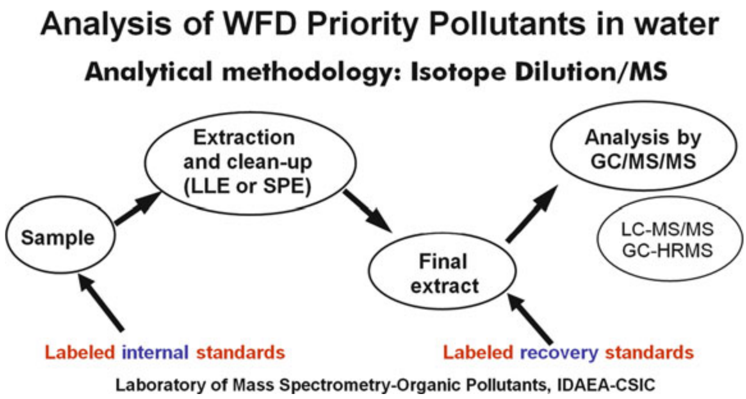

Fig. 4 Scheme of analytical methodology for the analysis of priority pollutants in water. *Source*: Laboratory of Mass Spectrometry-Organic Pollutants

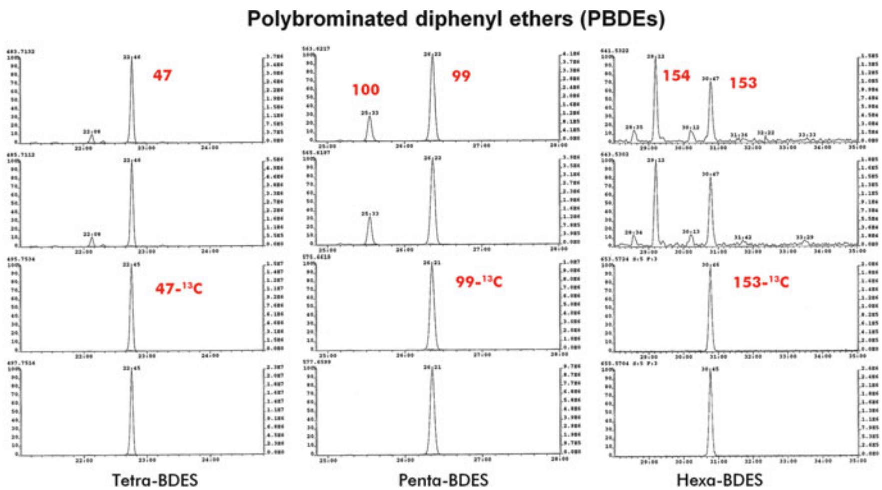

Fig. 5 Example of use of IDMS for analyzing PBDEs. Profile of a marine sediment obtained by GC/HRMS ($R = 10{,}000$), with the congeners and its isotope labeled analogous. *Source*: Laboratory of Mass Spectrometry-Organic Pollutants

proposed could follow the scheme shown in Fig. 4, with the addition of labeled standards at the beginning of the method and at the end to check the efficiency of the extraction. Figure 5 shows an HRMS chromatogram obtained working with IDMS, where there are the signals for native and labeled congeners.

3.3 Analytical Difficulties for Existing and "New" Priority Substances

We would like to remark the specific analytical difficulties of some compounds, many of them already described in recent literature.

3.3.1 Polybrominated diphenyl ethers (PBDEs)

Required LOQs for PBDEs according to EQS on 2008/105/EC [2] are with difficulty achieved in routine laboratory conditions [18], but the new directive [3] has included more acceptable EQS (140 ng/L and 14 ng/L, respectively, for inland and other surface waters). In addition, EQS for biota has been determined (0.0085 μg/Kg).

With all substances that an EQS for biota is established, the Directive [3] recommends the monitoring in this matrix.

3.3.2 Polycyclic Aromatic Hydrocarbons (PAHs)

The PAHs have focused on the B[a]Pyrene as a marker, with EQS0.17 ng/L (LOQ required 0.051 ng/L) and 5 μg/kg in biota. The lowest LOQs for water analysis achieved with methods applied by EU Member States are not sufficient of compliance monitoring in waters [14]. Large-volume water sampling is proposed for increasing method sensitivity [18].

3.3.3 Endosulfan

Although endosulfan is a common analyzed pesticide, the LOQ required, particularly AA-EQS for coastal waters, is not easy to achieve with routine methods [18].

3.3.4 Short-Chain Chlorinated Paraffins (SCCPs)

The difficulties in the analysis of SCCP reside in the highly complex nature of commercial formulations; the numerous physical, chemical, and biological processes after use; and the lack of certified chemical standards [18]. There is a variety of approaches to analyze SCCPs in environmental samples [18]. A validated procedure for routine monitoring of SCCPs was needed in fulfilling the technical specifications [4]. The ISO/DIS 12010 describes a method using gas chromatography/mass spectrometry (GC-MS) and electron capture negative ionization (ECNI) [19]. The method was validated and allows an analysis of SCCP under routine conditions for laboratories [20].

3.3.5 Perfluorinated Compounds (PFC)

In spite of the improvement of the quality in PFC analysis [18], the LOQ achieved with the ISO method 25101 is not sufficient for compliance monitoring in inland and coastal surface waters [14]. To reach LOQs is difficult partly due to blank problems that force to an accurate methodology [21]. However, the EQS for biota is considered more viable.

3.3.6 Cypermethrin

One of the most difficult "new" PS is cypermethrin, with an EQS of 80 pg/L (8 pg/L in coastal waters). Although extracting large-volume samples and a strong pre-concentration, sufficiently low LOQs could not be reached [13]. To reach LOQs in the low pg/L concentration range is extremely difficult, if not impossible with current methods [14].

3.3.7 Heptachlor/Heptachlor Epoxide

LOQs reported by literature are not sufficient for compliance monitoring (60 fg/L in inland surface waters and 3 fg/L in coastal waters) [14]. These PS can be analyzed in biota (LOQ, 2.01 pg/g) and very difficult to reach even with high-resolution mass spectrometry (HRMS).

Other substances that present problems to reach LOQs required are:

- *Aclonifen*: 36 ng/L (3.6 ng/L coastal waters)
- *Bifenox*: 3.6 ng/L (0.36 ng/L coastal waters)
- *Cybutryne*: 0.75 ng/L
- *Quinoxyfen*: 45 ng/L (4.5 ng/L coastal waters)
- *Terbutryn*: 19.5 ng/L (1.95 ng/L coastal waters)
- *Dichlorvos*: 0.18 ng/L (18 pg/L coastal waters)
- *Dicofol*: 0.39 ng/L (9.6 pg/L coastal waters)

4 Summary

The implementation of WFD in its entirety is not, in our opinion, an easy work. The requirements in terms of EQS and LOQs require the use of most advanced instrumentation, not available in many cases to all laboratories. It is a necessary exercise, as organized by JRC, to harmonize methods and results that bring to establish adequate and realistic EQS and let intercomparison of results between member states.

The watch list mechanism is a good approach to collect high-quality information of emerging pollutants and in consequence set reasonable EQS for which could be included in future revisions of the Directive. Even today, there are many substances which LOQ is difficult if not impossible to reach. Some strategies have been proposed to achieve lower LOQs, for example, extracting higher volumes of water; however, these methodologies are not very useful for WFD compliance monitoring; they are very work intensive and very costly [14]. Although the LOQs obtained by each laboratory depend on their *state of the art* and its instrumentation, they give us a plausible approximation of the outstanding challenges as well as the inconsistencies in the Directive's proposals.

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Pollutants of Emerging Concern in Rivers of Catalonia: Occurrence, Fate, and Risk

Antoni Ginebreda, Sandra Pérez, Daniel Rivas, Maja Kuzmanovic, and Damià Barceló

Abstract The present chapter provides a review of the occurrence, fate, and risk associated to different families or emerging and priority organic micropollutants in the rivers of Catalonia. Compounds belonging to diverse groups such as industrial compounds, perfluoroalkyl substances, pesticides, halogenated flame retardants, pharmaceuticals and hormones, personal care products, and illegal drugs, as well as their transformation products, are examined. Both emission levels from sewage systems and those found at the receiving water bodies are compared. Potential fate and transformation of the parent compounds is taken into consideration. Finally their environmental risk in terms of the associated ecotoxicity with respect to three trophic levels (*Daphnia*, algae, and fish) as recommended by the WFD is assessed. This prioritization exercise allows identifying those micropollutants that are more relevant in Catalonian Rivers.

Keywords Ecotoxicity, Emerging contaminants, Prioritization, Risk assessment, Transformation products, Water Framework Directive

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Contents

1 Introduction

Chemical pollution is widely recognized as one of the major threats to aquatic systems [1]. It is a direct consequence of the massive use of chemicals by our technological society (Fig. 1). Thus, for instance, in the European Union, there are more than 100,000 registered chemicals listed by EINECS (the European Inventory of Existing Commercial Chemical Substances) that may be considered of common industrial and/or domestic use (Fig. 1). Up to 30,000 of those compounds may be considered of concern and are subjected to the new REACH legislation [2]. Depending on their physical-chemical properties, amounts produced, and mode of use, many of these compounds may enter the natural waters through sewage water discharge, surface runoff from agricultural fields, atmosphere deposition, accidental spills, etc. On the other hand, many of these compounds are not properly eliminated by conventional wastewater treatment plants and are being continuously released as a part of the effluent. Contrastingly, up to now only a small fraction (i.e., 45 compounds) of those potential pollutants is covered by the so-called WFD list of "priority pollutants" (Directive 2013/39/UE) [3] for which Environmental Quality Standards (EQS) are fixed. Even though the list of priority pollutants is subjected to periodic update, the imbalance between the numbers of regulated and potential pollutants seems still disproportionate. On the other hand, the WFD states the obligation to identify pollutants of regional or local importance and provide EQS, monitoring schemes and management measures for them. This means that Member States need to decide which of the candidate substances for further investigation are and which of them are selected (prioritized) to be declared as river basin-specific pollutants [4, 5].

As a whole one can conclude that chemicals that are being routinely monitored on a regular basis by the responsible water authorities cover only a small fraction of all the chemicals present in the environment [6]. Many unregulated, emerging contaminants that are identified in the aquatic environment may have a significant impact on the aquatic ecosystems and require special attention [7]. Albeit, they are

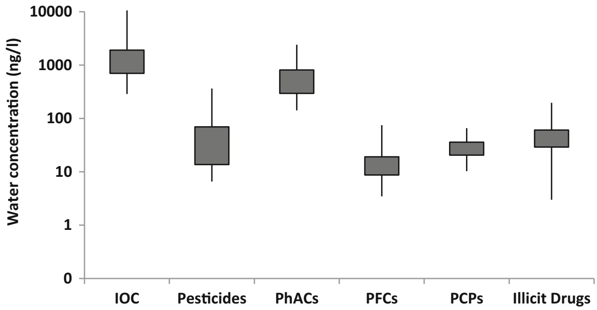

Fig. 1 Water phase concentration ranges of different emerging contaminant classes in the Llobregat River basin (IOCs, industrial organic compounds; PhACs, pharmaceuticals and hormones; PFCs, perfluoroalkyl compounds; PCPs, personal care products). For each family, whiskers correspond to quartiles 100 and 25 and upper and bottom boxes bounds to quartiles 75 and 50, respectively

usually present in very low concentrations (i.e., from pg/L to μg/L) because the improvement of analytical techniques, number, and frequency of detections of emerging contaminants is continuously growing [8]. It is worth noting that emerging environmental contaminants are not necessarily new chemicals. Actually they may be substances that have been present in the environment for a long time but whose potentially adverse effects on human health and the environment are only recently being recognized [4]. A further feature characterizing these compounds is related to the fact that owing to their high consumption and continuous introduction thereof into the environment, they need not be necessarily persistent to cause adverse effects [9].

As a consequence, the occurrence of emerging contaminants in the aquatic environment has been the object of many studies carried out in the context of research projects or as part of investigative monitoring by the responsible water authorities. In the next sections, we provide an overview of those performed in the Catalan River District [10, 11]. It is worth noting that among all the rivers located in the Catalan district, the Llobregat River deserved special attention, thus concentrating a major part of the studies about emerging contaminants carried out in the district [11]. Two reasons explain this choice: first of all, it can be considered a case study representative of Mediterranean rivers in terms of hydrologic behavior and climate conditions and, second, because it is located close to the Barcelona area where most of the population and industry of the region is settled, thus playing a key role in the local water cycle both as supply source and effluent receiving body.

2 Occurrence of Emerging Pollutants

There are several groups or families of emerging pollutants which result from either industrial, household, or agriculture use. They include halogenated flame retardants, water disinfection by-products, gasoline additives, hormones and other endocrine disruption products, pharmaceuticals, personal care products, perfluoroalkyl substances, illegal drugs, polar pesticides, organometallics, siloxanes, surfactants, plasticizers, antioxidants, corrosion inhibitors, and a variety of industrial compounds as well as other new materials recently identified as pollutants of concern such as nanomaterials or microplastics [12].

Table 1 provides some selected references concerning the different studies that have been carried out in the Catalan River District. They encompass those referred to the receiving water bodies (rivers) as well as those addressed to characterize the emissions from WWTPs. As mentioned, the majority of them were carried out in the Llobregat basin, followed by the Ebro and in much less extent in others (Ter, Besós, etc.). In general most of the studies reported are focused on the specific families of pollutants, being the comparison among the various ones not straightforward.

The most comprehensive and recent survey of emerging contaminants available in the Catalan River District was carried out in the Llobregat River in the context of the national-funded research project SCARCE-CONSOLIDER [59]. In the course of this project, the occurrence (concentration levels) of 199 organic micropollutants belonging to different groups of priority and emerging contaminants were measured in the main river and tributaries along two campaigns (2010 and 2011) [13]. They included pesticides (39), pharmaceuticals and hormones (89), perfluoroalkyl substances (PFAS) (21), industrial compounds (14), drugs of abuse (19), and personal care products (17). 158 out of the 199 compounds analyzed showed nonzero levels. As regards the various substance classes concerned, industrial compounds (10^3–10^4 ng L^{-1}) were the dominant group both in terms of whole class and on a single-compound basis (Fig. 1).

Pharmaceuticals (10^1–10^3 ng L^{-1}) were the second one, while personal care products, pesticides, perfluoroalkyl substances, and illegal drugs showed concentrations approximately one order of magnitude less (Fig. 1). Maximum and mean concentrations measured in the water phase for the individual micropollutants monitored are reported in Table 2.

Industrial compounds are dominated by triazoles (benzotriazole and tolyltriazole) employed in industry as anticorrosion agents (concentrations in the range of 500 to 5,000 ng L^{-1}), followed by bisphenol A (90–650 ng L^{-1}), some trialkyl phosphates used as flame retardants, and the group of alkylphenols (nonylphenol and octylphenol) and some related ethoxylated derivatives, all of them resulting from the biodegradation of the corresponding polyethoxylated compounds used as tensioactives, both in industry and household (range 10–1,000 ng L^{-1}). Nonylphenol monocarboxylate (NP1EC) is the dominating compound in that class (ca. 200–1,000 ng L^{-1}). It is worth mentioning that both

Table 1 Selected references related to studies about emerging and priority contaminants performed in the Catalan River District

Compound Class	Environment	River Basin	References
Various	River	Ebro	[13]
		Llobregat	[14]
		Ter	[15, 16]
	WWTP	Ebro	[13]
		Ter	[15]
		Other rivers	[17, 18]
Halogenated flame retardants	River	Ebro	[19–22]
		Llobregat	[19–21, 23]
	WWTP	Ebro	[19]
		Other rivers	[17]
Endocrine disruptors	River	Ebro	[13, 24, 25]
		Llobregat	[13, 24–26]
		Ter	[15]
	WWTP	Llobregat	[26–28]
		Other rivers	[16]
		Ter	[15]
Illicit drugs	River	Ebro	[13, 29, 30]
		Llobregat	[13, 27, 28]
	WWTP	Ebro	[29, 30]
		Llobregat	[27, 28, 31, 32]
Perfluoroalkyl substances	River	Ebro	[13, 24]
		Llobregat	[13, 24, 33, 34]
	WWTP	Llobregat	[33, 34]
Personal care products	River	Ebro	[13]
		Llobregat	[13, 35, 36]
	WWTP	Other rivers	[17, 35, 36]
Pesticides	River	Ebro	[13, 37–42]
		Llobregat	[13, 27, 28, 43–46]
	WWTP	Llobregat	[27, 28]
		Other rivers	[17, 47]
Pharmaceuticals	River	Ebro	[13, 24, 48–51]
		Llobregat	[13, 24, 26, 45, 52–54]
	WWTP	Ebro	[49–51]
		Llobregat	[26–28]
		Other rivers	[17, 18, 55, 56]
Nanomaterials (fullerenes)	River	Llobregat	[57]
	WWTP	Llobregat	[57]
Siloxanes	River	Llobregat	[58]
	WWTP	Llobregat	[58]

Table 2 Mean and maximum water concentrations of emerging contaminants classified per classes found in the Llobregat River basin in 2010–2011 (project SCARCE-CONSOLIDER [13, 59])

Compound	Concentration (ng L^{-1})[a]	
	Mean	Max
Industrial organic compounds		
Octylphenol (OP)	7.04	84.73
Octylphenol diethoxylate (OP2EO)	7.35	32.84
Octylphenol monocarboxylate(OP1EC)	0.04	1.25
Octylphenol monoethoxylate (OP1EO)	bld	bld
Nonylphenol (NP)	15.58	116.34
Nonylphenol monoethoxylate (NP1EO)	bld	bld
Nonylphenol diethoxylate (NP2EO)	41.50	287.67
Nonylphenol monocarboxylate (NP1EC)	212.18	989.53
Tolyltriazole (TT)	537.91	7,017.67
Tris(2-chloroethyl) phosphate (TCEP)	31.44	232.40
Tris(butoxyethyl) phosphate (TBEP)	81.45	315.08
Tris(chloroisopropyl) phosphate (TCCP)	218.93	1,117.27
1H-Benzotriazole (BT)	317.40	1,622.99
Bisphenol A (BPA)	89.21	649.35
Pesticides		
3-Hydroxycarbofuran	bld	bld
Acetochlor	bld	bld
Alachlor	bld	bld
Atrazine	0.59	6.44
Azinphos-ethyl	0.47	3.43
Azinphos-methyl	0.55	8.69
Buprofezin	0.24	4.38
Carbofuran	1.28	6.75
Chlorfenvinphos	0.24	3.48
Chlorpyrifos	4.63	13.65
Deisopropylatrazine	bld	bld
Desethylatrazine	bld	bld
Diazinon	5.72	35.77
Dichlofenthion	bld	bld
Dimethoate	3.11	71.91
Diuron	14.67	159.53
Ethion	0.46	7.10
Fenitrothion	1.69	47.39
Fenoxon	bld	bld
Fenoxon sulfone	0.17	1.76
Fenoxon sulfoxide	bld	bld
Hexythiazox	0.93	24.00
Imazalil	1.59	6.33
Imidacloprid	9.04	66.53

(continued)

Table 2 (continued)

Compound	Concentration (ng L^{-1})[a]	
	Mean	Max
Isoproturon	1.53	9.60
Malathion	0.79	9.13
Methiocarb	0.21	3.23
Metolachlor	1.00	12.96
Molinate	bld	bld
Omethoate	bld	bld
Parathion-ethyl	bld	bld
Parathion-methyl	bld	bld
Prochloraz	0.35	9.87
Propanil	bld	bld
Propazine	0.90	8.77
Pyriproxyfen	0.06	1.72
Simazine	3.66	45.77
Terbutryn	2.20	23.37
Tolclofos-methyl	bld	bld
Pharmaceuticals and hormones		
Phenazone	1.14	9.53
Propyphenazone	1.96	24.40
Oxycodone	0.83	4.35
Codeine	3.95	44.07
Hydrocodone	0.31	3.56
Acetaminophen	23.02	142.89
Ibuprofen	45.29	179.31
Indomethacin	6.70	63.72
Diclofenac	28.80	280.00
Ketoprofen	33.55	153.09
Naproxen	20.41	90.53
Piroxicam	0.63	4.32
Meloxicam	0.06	1.58
Tenoxicam	bld	bld
Erythromycin	0.88	12.66
Azithromycin	2.36	12.20
Clarithromycin	1.76	28.33
Tetracycline	0.61	17.01
Sulfamethoxazole	2.75	41.91
Trimethoprim	11.85	150.43
Metronidazole	0.92	10.07
Metronidazole-OH	1.63	6.20
Ofloxacin	4.82	43.55
Ciprofloxacin	1.61	20.00
Cephalexin	2.45	5.08

(continued)

Table 2 (continued)

Compound	Concentration (ng L^{-1})[a]	
	Mean	Max
Bezafibrate	3.48	24.55
Gemfibrozil	71.54	302.67
Pravastatin	1.44	7.82
Fluvastatin	0.37	3.90
Atorvastatin	2.53	5.75
Loratadine	0.78	4.29
Desloratadine	4.45	10.27
Ranitidine	1.64	18.44
Famotidine	0.00	bld
Cimetidine	1.25	19.42
Atenolol	19.54	331.58
Sotalol	10.32	223.81
Metoprolol	16.58	295.56
Propanolol	2.36	12.41
Nadolol	2.91	4.82
Enalapril	1.04	10.22
Enalaprilat	19.66	91.20
Diltiazem	4.19	31.80
Irbesartan	15.50	141.10
Losartan	17.85	126.88
Valsartan	62.99	698.90
Torasemide	1.47	9.43
Fluoxetine	2.15	9.46
Norfluoxetine	2.55	4.42
Paroxetine	5.52	12.46
Diazepam	2.30	35.51
Lorazepam	18.44	187.87
Alprazolam	0.72	4.98
Carbamazepine	7.41	64.04
Sertraline	10.95	144.87
Citalopram	3.22	31.83
Venlafaxine	12.55	127.62
Olanzapine	8.02	20.19
Trazodone	3.36	34.27
Albendazole	1.72	5.11
Thiabendazole	2.35	12.92
Levamisole	4.90	37.85
Dimetridazole	3.14	18.39
Ronidazole	bld	bld
Xylazine	0.17	1.10
Carazolol	2.67	6.43

(continued)

Table 2 (continued)

Compound	Concentration (ng L^{-1})[a]	
	Mean	Max
Azaperone	0.26	7.18
Azaperol	0.08	2.19
Dexamethasone	0.76	4.85
Hydrochlorothiazide	128.28	793.33
Furosemide	34.20	296.47
Glibenclamide	0.33	4.61
Warfarin	0.51	1.20
Acridone	4.04	42.73
Tamsulosin	0.23	0.67
Salbutamol	1.40	16.82
Amlodipine	1.80	23.52
Clopidogrel	2.86	17.98
Iopromide	67.58	1,370.37
Diethylstilbestrol (DES)	bld	bld
Estradiol (E2)	0.62	2.17
Estradiol 17-glucuronide (E2-17G)	bld	bld
Estriol (E3)	0.20	5.69
Estriol 16-glucuronide (E3-16G)	bld	bld
Estriol 3-sulfate (E3-3S)	0.46	12.78
Estrone (E1)	0.92	6.21
Estrone 3-glucuronide (E1-3G)	0.14	4.03
Estrone 3-sulfate (E1-3S)	bld	bld
Ethinyl estradiol (EE2)	bld	bld
Caffeine	208.99	1,220.90
Perfluorinated compounds		
L-PFOS	117.70	2,708.71
L-PFHxS	3.71	33.18
PFBA	10.15	111.17
PFPeA	0.51	5.26
PFHxA	1.27	25.15
PFHpA	3.16	30.93
PFOA	11.10	146.40
PFNA	2.00	52.36
i,p-PFNA	0.02	0.19
PFDA	2.34	54.31
PFUdA	0.32	3.65
PFDoA	0.56	7.92
PFTrDA	0.91	9.75
PFTeDA	0.77	7.59
PFHxDA	0.16	4.25
PFODA	bld	bld

(continued)

Table 2 (continued)

Compound	Concentration (ng L^{-1})[a]	
	Mean	Max
L-PFBS	2.82	25.69
L-PFHpS	bld	bld
L-pPFNS	1.23	12.00
L-PFDS	0.06	0.82
PFOSA	bld	bld
Personal care products		
2,2′-Dihydroxy-4-methoxybenzophenone (DHMB)	bld	bld
4,4′-Dihydroxybenzophenone (4DHB)	5.97	153.00
4-Hydroxybenzophenone (4HB)	0.06	1.70
4-Methylbenzylidene camphor (4MBC)	1.16	9.30
Benzophenone-1 (BP1)	1.69	15.40
Benzophenone-2 (BP2)	bld	bld
Benzophenone-3 (BP3)	3.49	44.10
Ethyl 4-aminobenzoate (Et-PABA)	bld	bld
Ethylhexyl dimethyl PABA (OD-PABA)	0.18	2.10
Ethylhexyl methoxycinnamate (EHMC)	3.39	41.00
Octocrylene (OC)	2.18	27.00
Triclocarban	bld	bld
Triclosan	1.04	13.63
Propylparaben	3.58	20.21
Benzylparaben	0.99	6.69
Ethylparaben	4.10	40.69
Methylparaben	5.38	50.94
Illicit drugs		
(−)-9-THC	bld	bld
(±)-11-hydroxy-THC	bld	bld
(±)-11-nor-9-carboxy-9-THC	bld	bld
(±)-Amphetamine	bld	bld
(±)-EDDP perchlorate	8.63	49.50
(±)-MDMA	8.76	56.80
(±)-Methadone hydrochloride	3.51	20.00
(±)-Methamphetamine	0.09	0.38
1S,2R (+)-Ephedrine	12.80	88.60
2-oxo-3-hydroxy LSD	bld	bld
6-Acetylmorphine	bld	bld
Benzoylecgonine	11.06	44.00
Cannabidiol	bld	bld
Cannabinol	bld	bld
Cocaethylene	bld	bld
Cocaine	3.62	23.80
Heroin	bld	bld

(continued)

Table 2 (continued)

Compound	Concentration (ng L^{-1})[a]	
	Mean	Max
LSD	bld	bld
Morphine	0.49	3.02

bld below limit of detection

nonylphenol and octylphenol are included in the WFD priority list (*Directive 2013/39/UE*) due to their proved endocrine disrupting activity.

Perfluoroalkyl substances are largely used by industry and are present in consumer products as well. Perfluorooctane sulfonate (PFOS) and perfluorooctane carboxylic acid (PFOA) are of much concern. Remarkably PFOS has been recently included in the list of priority compounds of the WFD (*Directive 2013/39/UE*). Among the perfluoroalkyl compounds monitored, these were too the most relevant, showing maximum concentration levels of up to 2,700 ng L^{-1} and 150 ng L^{-1}, respectively, for PFOS and PFOA.

Occurrence of pharmaceuticals is closely related to the population distribution. Thus, 81 pharmaceuticals and hormones out of the 89 analyzed belonging to different therapeutical classes have been positively detected in the Llobregat River basin, being their corresponding mean concentrations up to 100 ng L^{-1} depending on the compounds. Those showing higher levels were the diuretic hydrochlorothiazide and the anti-inflammatories ibuprofen, diclofenac, and ketoprofen, followed by the antilipidemic agent gemfibrozil and the antihypertension agent valsartan. Other anti-inflammatories such as acetaminophen, naproxen, and codeine; the antilipidemic bezafibrate; the beta-blockers atenolol, sotalol, metoprolol, and nadolol or the ACE inhibitor enalaprilat; the antibiotics ofloxacin and trimethoprim; or psychiatric drugs carbamazepine and lorazepam follow next. In general, they are consistent with their respective consumption. Estrogenic hormones such as estradiol (E2), estriol (E3), and its sulfate conjugate and estrone are found at concentration levels in the range of 1–10 ng L^{-1}.

Despite that pesticides are not the most relevant group in terms of concentration (average ranges per single compound of 1–10 ng L^{-1} with peaks up to 100 ng L^{-1} for few of them) (Table 1), from the environmental point of view, they are certainly the group causing more risk to the aquatic ecosystems due to their inherent toxic properties (see next Section "Risk Assessment and Prioritization"). Actually 25 out of the 39 pesticide compounds (insecticides and herbicides) analyzed were positively identified in the Llobregat basin, being the most relevant the herbicides diuron, terbutryn, and simazine (included in the WFD priority list) and the insecticides diazinon, dimethoate, fenitrothion, and malathion.

Personal care products monitored included UV filters (11), disinfectants (2), and antioxidants (parabens) (4). Top compounds are methyl- and propylparabens, triclosan (disinfectant), and UV filters benzophenone-3, 4,4′-dihydroxybenzophenone (4DHB), and ethylhexyl methoxycinnamate (EHMC), all of them showing maximum concentrations in the range of ca. 10–100 ng L^{-1}.

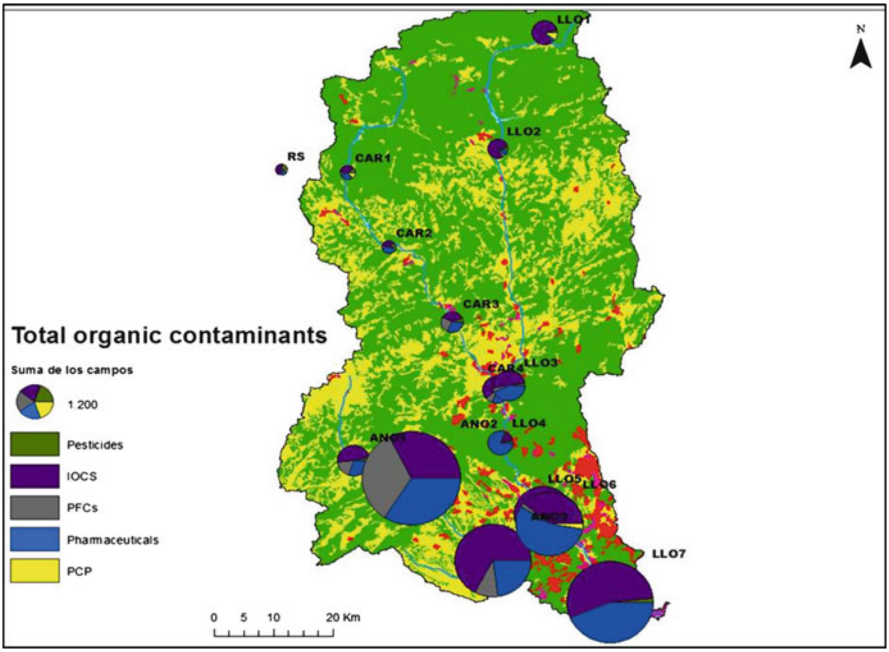

Fig. 2 Distribution of emerging contaminant classes along the Llobregat basin (circle sizes are proportional to overall concentrations)

Drugs of abuse analyzed included 19 substances (parent compounds and metabolites) corresponding to several subfamilies such as cannabinoids, lysergic acid derivatives, cocainics, amphetaminics, and opioids. Among the compounds detected, the most relevant compounds were amphetaminics EDDP, MDMA (ecstasy) and ephedrine (used also as pharmaceutical), methadone, cocaine, and its metabolite benzoylecgonine.

Finally it is worth mentioning the case of caffeine, which strictly speaking is not included in none of the abovementioned families. It is originated by population consumption of coffee, tea and soft drinks and discharged into river from WWTP where it is only partially eliminated. Caffeine is thus a convenient tracer of urban pollution. Even though it is not expected to cause acute effects in the aquatic ecosystem, it is frequently detected at variable concentrations (mean of 200 ng $L^{-1}$and maximum of 1200 ng L^{-1}).

As regards the spatial distribution of pollution along the basin (Fig. 2), as expected it tends to increase downstream, particularly in the surroundings of the Barcelona where most of the industry and population of the basin is concentrated (up to 4,000,000 inhabitants, of which approximately 1,500,000 discharge their treated wastewater in the basin). Nevertheless, there are some other “hot spots” located in other sites, notably the Anoia tributary nearby the town of Igualada where industrial activity is relevant as well.

3 Fate and Transformation of Emerging Pollutants in Rivers

3.1 Fate of Emerging Pollutants

Emerging pollutants can reach the surface waters via different routes and are then transported, distributed, and transformed (Fig. 3). The physicochemical properties of the pollutants, such as water solubility, vapor pressure, and polarity determine their behavior in rivers. The major sources of environmentally relevant contaminants of emerging concern are primarily WWTP effluents which receive inputs from households and industry and secondarily terrestrial runoffs (roofs, pavement roads, agricultural land) and also the direct application (in case of pesticides) as well as atmospheric deposition. A special group of emerging pollutants are human and veterinary pharmaceuticals which after consumption may enter in the WWTPs already transformed [60]. To some extent pharmaceuticals and their human metabolites pass through the WWTP and consequently can enter rivers or surface waters. In addition, pharmaceuticals can reach surface waters by runoff from fields amended with digested sewage sludge or manure from farms. Another group of emerging pollutants which enter to surface waters from WWTPs are personal care products like fragrances which are discharged through shower waste. One of the groups with most proved potential adverse effects on the environment is alkylphenol ethoxylates (APEO) and nonylphenol (NP) because nonylphenol ethoxylates (NPEO) degrade to NP which presents endocrine disruption properties. They are nonionic surfactants that have been used extensively in cleaning products and industrial processes. More than 90% of APEO produced worldwide are NPEO. Nonylphenol and NPEO are commonly present in WWTPs due to their extensive domestic and industrial use, and as a result, APEO and nonylphenol are found in surface water, suspended particulate material, and sediments [61]. Pesticides are extensively used worldwide both in rural and urban applications, and they enter into

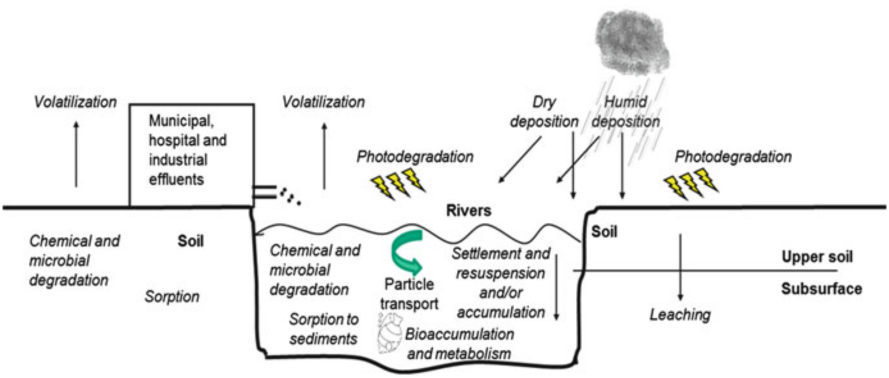

Fig. 3 Fate and transformation of organic micropollutants in the aquatic environment

surface waters after their application. Perfluorinated compounds (PFCs) are another relevant group of pollutants of emerging concern [62, 63]. This class includes perfluorooctane sulfonate and perfluorooctanoic acid in addition to a large number of other structurally related compounds. Because perfluorocarbons and perfluorosulfonic acids are very stable, they sooner or later turn up in the environment, especially in surface waters.

3.2 Natural Attenuation

Once the pollutants of emerging concern reach rivers, their concentrations may decrease by both natural and artificial processes (Fig. 3); the latter includes the reuse of surface water recharging aquifers for drinking water purposes. Natural processes can be classified as biological or physicochemical. Physical processes include physical dispersion and dilution which do not chemically alter the pollutant structure, but their concentrations may be mitigated one order of magnitude below the concentrations detected in the WWTP effluent [64]. These dilution processes occur mainly in areas with large rivers, which upon receiving wastewater contaminated with drug residues may dilute their concentration. In contrast, in a recent study comparing the concentration of pharmaceuticals in the treated WWTP effluent and the receiving surface waters of Ebro River, they were almost the same (concentrations of drugs were in the range of hundreds of ng L^{-1}) indicating almost no dilution of the WWTP effluent [65]. Volatilization is another important factor in the removal of organic compounds from a river process which depends on the physicochemical properties of the substance, mostly its vapor pressure. The pollutants end up into the air from resuspension process of particles found in the sediments/soils or sludge or directly from volatilization from water. Recent study shows the presence of drugs of abuse in particulate matter originating from resuspension into the air of these substances when they are used in the powder form [66]. The concentrations of cocaine ranged from 204 to 480 pg/m^3, tetrahydrocannabinol from 27 to 44 pg/m^3, amphetamine from 1.4 to 2.3 pg/m^3, and heroine from 9 to 143 pg/m^3 [66]. In addition, particulate matter from the sediment can transport pollutants of emerging concern along the river (Fig. 3). Recently, in a scientific paper, 31 drugs were detected in the Ebro River particulate from different classes of compounds, i.e., anti-inflammatories and analgesics followed the B-antagonists and antibiotics which were the most detected classes of compounds [65]. For example, in this study, maximum concentrations of paracetamol in particulate matter were 657 ng L^{-1} (the concentration was calculated in the particulate matter percolating 1 L of water), 442 ng $L^{-1}$of ibuprofen, and 95 ng $L^{-1}$of the antibiotic clarithromycin. In contrast, sediments presented lower concentrations of the target drugs, as in the case of acetaminophen which showed peak concentrations of 222 ng L^{-1} or ibuprofen (maximum concentration of 20.9 ng L^{-1}) or clarithromycin of 3.75 ng L^{-1} [65]. In this study the distribution of the drugs between the aqueous phase and the particulate matter was also calculated, showing that 30% of the 43 detected drugs were at measurable levels in the particulate phase. In this case, basic compounds ($pK_a > 7$) such as famotidine, timolol, and nadolol had a higher tendency to bind to the particulate phase [65].

Regarding the degradation of emerging pollutants in rivers, the two most important processes are photolysis and biodegradation. Photolysis is the breakdown of a substance by the effect of light. This *abiotic transformation process* can be direct photochemical degradation and/or by a wide array of indirect photochemical pathways, including reaction with singlet oxygen ($^1O_2$), hydroxyl radical ($^{\bullet}OH$), peroxy radicals ($^{\bullet}OOR$), photo-excited organic matter, and other reactive species [67]. To evaluate these processes in a river, usually laboratory studies are performed in a first stage, and then in the next step studies are conducted directly in the natural environment. For instance, this approach was used in the evaluation of the photolysis of antiviral oseltamivir and its human metabolite, oseltamivir ester, in water samples from the Ebro River [68]. To this end, first a photodegradation study of the two compounds in different aqueous matrices was carried out at lab scale and then was evaluated, and the photolysis of the two target compounds was assessed at natural scale. To this end, surface water samples were spiked individually with the prodrug oseltamivir ester and oseltamivir carboxylate and then photodegraded in a sunlight simulator (Suntest) allowing to identify several transformation products (TPs). Therefore, several surface water samples from the Ebro River were taken and analyzed for oseltamivir, its human metabolite, and their TPs. Of the TPs identified in the lab, two were detected in these samples providing evidence for photolysis and thus underpinning the importance of natural attenuation processes in rivers [9]. In another study on the photolysis of six iodinated X-ray contrast media in the Llobregat River, fewer TPs were detected in the natural river water than in the sunlight simulator (11%). In the photolysis experiments, 108 TPs were detected. Then real samples were taken in the Llobregat River, and only 11 priority TPs were detected in the river water samples [69]. Photolytic reactions are often complex pathways leading to multiple reaction products, TPs. These TPs can be more toxic than the parent compound [70] or retain the pharmacological properties (i.e., antibiotic activity) as demonstrated for some dehydrated products of tetracyclines [71] and photodegradation products of the fluoroquinolone antibiotic ofloxacin [72].

Another important process for the natural attenuation of pollutants of emerging concern in rivers are catabolic biodegradation processes involving microorganisms, algae, yeast, and fungi which may partially or completely decompose organic compounds. Like in the phototransformation processes, the biodegradation TPs can be more toxic than the parent compound as in the case of NPEO; their degradation yields nonylphenol which is persistent in the aquatic environment and toxic to aquatic organisms [73].

To date, no studies of the biodegradation of emerging pollutants in the Spanish rivers have been published; however, reports are available from other countries like the USA [74]. In this study, declining levels of the chiral drug atenolol along a river were observed. As this went along with a change in the enantiomeric fraction, biodegradation was postulated, while photodegradation was ruled out. Many organic compounds are biodegraded by organisms that utilize them as energy source. Another important biodegradation process is cometabolism in which an organic compound is modified but not utilized for growth [75]. Whereas some compounds can evade photochemical reactions because they are not exposed to sunlight (e.g., when they are adsorbed onto particles or in the subsurface), microbial transformation processes constitute the dominant attenuation mechanism of emerging compounds. Plants and animals have some capabilities of detoxifying or excreting contaminants after uptake; however, accumulation in adipose tissue or the lack of appropriate enzyme systems necessary for biotransformation can hamper elimination of the contaminants. For instance, although PFCs are characterized by their high stability in the environment, they are subject to metabolism/degradation that leads to the formation of different metabolites [76, 77].

4 Risk-Based Prioritization of Organic Microcontaminants

Risk is broadly defined as the combination (i.e., product) of a probability of occurrence of some event by its associated hazard effects:

$$\text{Risk} = \text{Occurrence} \times \text{Adverse Effects}.$$

Correspondingly, the risk assessment process may be defined as the set of procedures aiming to identify hazards and to quantify the associated risk (in our case, related to chemicals) concerning human health and/or ecosystems impairment. In the case of the environmental risk posed by chemicals, "adverse effects" are related to the intrinsic harmful properties of each compound [5], typically persistence, bioaccumulation, and toxicity, being the latter the most relevant. On the other hand, "occurrence" is associated to its environmental exposure, usually expressed in terms of environmental concentration, which in turn can be either measured (MEC) or predicted (PEC) through modeling. Different risk assessment approaches have been developed in order to identify and rank compounds of environmental concern for both regulatory and monitoring purposes.

4.1 Definition of Chemical Risk: The Toxic Unit Approach

To assess the environmental risk of detected compounds from an ecotoxicological perspective, the toxic unit (TU) approach [78] is commonly used. TU is defined as the ratio of the compound's measured concentration (C_i) with respect to a certain toxicity reference value (Eq. 1):

$$\mathrm{TU}_{i\ (\mathrm{algae},\ \mathit{Daphnia}\ \mathrm{sp.},\ \mathrm{fish})} = \frac{C_i}{C_i(\mathrm{ref})}, \tag{1}$$

where TU_i is the toxic unit of a compound i corresponding to a measured concentration C_i (typically in ng L^{-1}) in the water phase and C_i(ref) is an ecotoxicity reference concentration. Typically EC50 or LC50 (effect or lethal concentration for 50% of individuals) for standard test organisms is used for acute risk, whereas PNEC (predicted no-effect concentration) is preferred for chronic risk estimation. In the later case, TU are commonly referred in the literature as "hazard quotients" (HQ). Correspondingly TU $\geq$ 1 (or HQ $\geq$ 1) would indicate a situation of potential risk of either acute or chronic ecotoxicity, respectively. In order to be representative from the ecological point of view, TU for different trophic levels should be calculated. Following the recommendations of the WFD algae, *Daphnia* sp. and fish are usually used.

4.2 Multichemical Risk Assessment

In real-world scenarios, contaminants rarely occur alone. Instead, they usually appear as mixtures of many compounds being their combined effects difficult to predict (i.e., synergies or antagonistic effects may take place). Therefore, toxicological effects caused by mixtures must be taken into consideration in RA studies. Mixture toxicity is a complex question and is a topic of active research. Interested readers are referred to recently published reviews [79, 80]. Specifically, in aquatic ecotoxicology, two different conceptual models, respectively, known as concentration addition (CA) [81] and *independent action* (IA) [82], are considered to describe general relationships between the effects of single substances and their corresponding mixtures, for similarly and dissimilarly acting chemicals, respectively [83]. The concentration addition model is founded on the assumption that mixture components each possess a similar pharmacological mode of action and thus is most applicable for toxic substances that have the same molecular target site .The alternative model of independent action or response addition assumes that mixture components possess dissimilar modes of action, interacting with different target sites, leading to a common toxicological endpoint via distinct chains or reactions within an organism.

The mixture effects for both CA and IA modes of action are respectively given below [80]:

Concentration addition (CA):

$$\mathrm{EC50}_{\mathrm{Mix}} = \sum_i \frac{c_i}{\mathrm{EC50}_i}, \tag{2}$$

where c_i is the concentration of component i, $\mathrm{EC50}_i$ is the toxicity of compound i expressed as EC50, and $\mathrm{EC50}_{\mathrm{Mix}}$ indicates the toxicity of the whole mixture expressed as EC50. $\mathrm{EC50}_{\mathrm{Mix}}$ is often referred as "toxic unit summation" (TUS) and the individual terms "toxic units" (TU) [78], Eq. (1) thus becoming:

$$\mathrm{TUS} = \sum_i \mathrm{TU}_i. \tag{3}$$

Independent action (IA):

$$E(c_{\mathrm{Mix}}) = 1 - \prod_{i=1}^{n} [1 - E(c_i)], \tag{4}$$

where $E(c_{\mathrm{Mix}})$ indicates the effect of a mixture of n-compounds, c_i is the concentration of the ith compound, and $E(c_i)$ is the effect of that concentration if the compound is applied singly.

Whereas both models have been proved acceptable if the corresponding mechanistic assumptions are fulfilled, since exact modes of action are often unknown for many compounds, both CA and IA must be regarded as two special extreme cases [84, 85] defining a frame where real values are contained. In practice, both models have been more or less successfully applied, being the results obtained with both of them not very different, with CA tending to overestimate and IA to underestimate toxicity in controlled experiments [86, 87]. For both simplicity of calculation and precautionary reasons, CA is usually the recommended method in a first-tier approach [80].

Table 3 summarizes a number of studies carried out in the different basins of the Catalan district.

4.3 Prioritization of Compounds of Environmental Concern

In some of the articles reported in Table 3, there are indications about what are the most relevant compounds in each specific case. However, in order to provide a more general approach for prioritization purposes, a "*ranking index*" (RI) was developed [13] which is a slight modification of that developed by von der Ohe et al. [88]. It is applicable to every compound on a certain area of study (here a river basin) and considers both the toxic units (TU) of the compound and its distribution in the area studied. To this end, six log TU ranges or classes were arbitrarily defined, which cover the typical occurrence values found in environmental samples. Rank frequencies f_x expressed as the fraction of sites (as a percentage) in the river basin where log TU of the compound belongs to the specific rank class x are determined in the Eq. (5):

Table 3 Summary of the published risk assessment and prioritization studies carried out in the Catalan River basin district (adapted and updated from [89])

Pollutants considered	Area/data source	Scope/remarks	References
Classical and priority contaminants	Llobregat River basin Data from the Catalan Water Agency collected for regulatory purposes	Risk assessment methodology: toxic units (TUS) based on *Daphnia* toxicity. TU values were compared with measured effects in transplanted *D. magna* individuals Compounds assessed: 7 types of contaminant assessed (copper, zinc, triazines, polycyclic aromatic hydrocarbons, organochlorine compounds, alkylphenols, organophosphorus pesticides), only the last group was likely to affect aquatic arthropods having similar sensitivities as *D. magna*	Damasio et al. [90]
Classical and priority contaminants	Catalonian river basins Data from the Catalan Water Agency collected for regulatory purposes (1997–2004)	Risk assessment methodology: COMMPS (Combined Monitoring-based and Modeling-based Priority Setting Scheme) A locally adapted list of priority pollutants at a regional scale and a new site pollution risk index for the relative comparison of the chemical pollution status of the investigated sites in the region are proposed	Teixidó et al. [91]
Classical and priority contaminants	Catalonian river basins Data from the Catalan Water Agency collected for regulatory purposes (2007–2008) (WFD)	Risk assessment methodology: exposure assessment with species sensitivity distribution (SSD) and mixture toxicity rules (CA and IA) were used to compute the multi-substances potentially affected fraction (msPAF) The total dataset of chemical monitoring carried out in Catalonia (Llobregat is included) between 2007–2008 (232 sampling stations and 60 pollutants) has been analyzed using sequential advanced modeling techniques. Data on concentrations of contaminants in water were pretreated in order to calculate the bioavailable fraction,	Carafa et al. [92]

(continued)

Table 3 (continued)

Pollutants considered	Area/data source	Scope/remarks	References
		depending on substance properties and local environmental conditions. The resulting values were used to predict the potential impact on aquatic biota of toxic substances in complex mixtures and to identify hot spots	
Classical and priority contaminants	Llobregat River basin Data from the Catalan Water Agency collected for regulatory purposes (2001–2004) (WFD)	Risk assessment methodology: integrated RA methodology for the classification of the ecological status (ES) based on the weight of evidence approach. It implements a fuzzy inference system that hierarchically aggregates a set of environmental indicators grouped into five lines of evidence, namely, biology, chemistry, ecotoxicology, physicochemistry, and hydromorphology. The ES is expressed as the membership degree to one or two contiguous WFD status classes. The method is implemented within a freeware GIS (Geographic Information System)-Based Decision Support System (DSS) developed as part of the MODELKEY project	Gottardo et al. [93, 94]
Classical, priority, and emerging organic contaminants	Water monitoring data collected at European level (Elbe, Scheldt, Danube, and Llobregat Rivers) Data from water authorities collected for regulatory purposes (WFD)	Five hundred compounds are classified in categories according to the type of assessment required. This allows water managers to focus on distinct actions according to the classification of a substance. To decide which compounds have the highest priority within each category, two indicators are proposed: (a) The *frequency of exceedance* (b) The *extent of exceedance* of the lowest predicted no-effect concentration (PNEC). For (a) maximum observed concentrations at each sampling site (MEC_{site}) are compared to the lowest PNEC, whereas for	Von der Ohe et al. [88]

(continued)

Table 3 (continued)

Pollutants considered	Area/data source	Scope/remarks	References
		(b) the 95th percentile of all MEC $_{site}$ were compared to the lowest PNEC values	
Emerging contaminants (pharmaceuticals, pesticides, alkylphenols, and heavy metals)	Water monitoring data collected in the Llobregat River middle and low basin CEMAGUA, AQUATOXIGEN, MODELKEY, and KEYBIOEFFECTS research projects	Risk assessment methodology: HQ based on independent action mode for invertebrates RA are compared to responses to field collected and transplanted invertebrate species (*Hydropsyche exocellata*, *Echinogammarus longisetosus*, and *Daphnia magna*) using up to 10 different endpoints including enzyme activities related with detoxication mechanisms (i.e., glutathione S transferase, catalase, esterases), the oxidative stress damage marker (lipid peroxidation), and individual responses (mortality, postexposure feeding rates) Estimated hazard indexes of measured pollutants indicated that pesticides and metals accounted for most of the predicted toxicity (>95%) in the most contaminated site and that the predicted toxicity of pharmaceuticals was marginal (<5%)	Damasio et al. [95]
Pharmaceutical and compounds	Water monitoring data collected in the Llobregat River basin MODELKEY research project	Risk assessment methodology: HQ based on concentration addition mode for fish, *Daphnia*, and algae Survey was carried out along three campaigns in 7 sampling points, located in the main river and in one of its tributaries (Anoia River). In each sample, 29 commonly used pharmaceuticals, belonging to different therapeutic classes (analgesics and nonsteroidal anti-inflammatory (NSAIDs), lipid regulators, psychiatric drugs, antihistamines, antiulcer agents, antibiotics, and β-blockers) have	Ginebreda et al. [96]

(continued)

Table 3 (continued)

Pollutants considered	Area/data source	Scope/remarks	References
		been determined HQ shows inverse correlation with Shannon-Wiener biodiversity index, being *Daphnia* the best one For the fish-based bioassay, the major contribution is due to gemfibrozil, followed by ibuprofen and diclofenac. Other compounds with significative effect are propyphenazone and bezafibrate. For *Daphnia*, major contributions are attributable to erythromycin, ibuprofen, and clofibric acid and, to a less extent, to diclofenac, acetaminophen, and sulfamethoxazole. Algae appear to be mostly dependent of sulfamethoxazole, followed by ibuprofen and gemfibrozil	
Pesticides	Water monitoring data collected in the Llobregat River middle and low basin VIECO research project	Risk assessment methodology: pesticide risk index for the surface water system (PRISW-1), based on the pesticide concentrations and their overall toxicity (estimated as TUS) against algae, *Daphnia*, and fish. It investigates the occurrence of 16 selected pesticides belonging to the classes of triazines, phenylureas, 30 organophosphates, chloroacetanilides, and thiocarbamates in surface waters from the Llobregat River and some tributaries (Anoia and Rubí) Application of the PRISW-1 index indicated that, although pesticides levels fulfilled the European Union Environmental Quality Standards (EQS) for surface waters, the existing pesticide contamination poses a low to high ecotoxicological risk for aquatic organisms The organophosphates diazinon	Köck-Schümeyer et al. [44]

(continued)

Table 3 (continued)

Pollutants considered	Area/data source	Scope/remarks	References
		and malathion and the phenylurea diuron are the major contributors to the overall toxicity	
Emerging contaminants (pharmaceuticals, illicit drugs, and estrogens)	Water monitoring data collected in the Llobregat River and low basin Data from the Catalan Water Agency (2008–2009)	Risk assessment methodology: HQ based on concentration addition mode for fish, *Daphnia*, and algae A total of 103 emerging contaminants belonging to the groups of pharmaceuticals (74), illicit drugs (17), and estrogens (12) were determined in river water samples during the water reuse campaign carried out in 2009 in the low Llobregat during a water reuse experiment Differences between river upstream and downstream to the discharge point were perceivable but not very significant, pharmaceuticals having higher contribution than illicit drugs. No relevant risks were identified	López-Serna et al. [27]
Classical and emerging contaminants	Sediment monitoring data collected at European level (Elbe, Scheldt, and Llobregat Rivers) MODELKEY research project	Risk assessment methodology: toxic units (TU) on the basis of acute toxicity to *Daphnia magna* and *Pimephales promelas* and multi-substance potentially affected fractions of species (msPAF) The toxicity of four polluted sediments and their corresponding reference sediments were investigated using a battery of six sediment contact tests representing three different trophic levels. The tests included were chronic tests with the oligochaete *Lumbriculus variegatus*, the nematode *Caenorhabditis elegans*, and the mud snail *Potamopyrgus antipodarum*, a subchronic test with the midge *Chironomus riparius*, an early life-stage test with the zebra fish *Danio rerio*, and an acute test with the	Tuikka et al. [97]

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Table 3 (continued)

Pollutants considered	Area/data source	Scope/remarks	References
		luminescent bacterium *Vibrio fischeri* The test battery could clearly detect toxicity of the polluted sediments. The msPAF and TU-based toxicity estimations confirmed the results of the biotests by predicting a higher toxic risk for the polluted sediments compared to the corresponding reference sediments but partly having a different emphasis	
Pesticides	Ebro River Delta	Monitoring study combining ecotoxicity measurements in water using three different bioassays and pesticide analysis in both water and shellfish has been carried out in this area in April–June 2008. Water and shellfish samples were collected at six selected sites, 2 located in the bays where seafood (mussels and oysters) are grown and 4 in the main draining channels discharging the output water from the rice fields into the bays Toxicity of the water samples has been evaluated using three standardized bioassays: 24–48 h immobilization of *Daphnia magna*, growth inhibition of *Pseudokirchneriella subcapitata*, and bioluminescence inhibition of *Vibrio fischeri*. Analysis of pesticides in water included 6 triazines, 4 phenylureas, 4 organophosphorus, 1 anilide, 2 chloroacetanilides, 1 thiocarbamate, and 4acid herbicides Results have shown individual pesticides concentrations in water above 100 ng/L for about 50% of the compounds investigated and total pesticide levels above 5 lg/L in the draining channels. A reasonable	Köck-Schümeyer et al. [39]

(continued)

Table 3 (continued)

Pollutants considered	Area/data source	Scope/remarks	References
		coherence has been observed between pesticide TUs (in water and shellfish), toxicity, and mortality episodes of shellfish for the different locations studied. Based on this observations, the pesticides suspected to be the main contributors to the total ecotoxicity are malathion and to a lesser extent diazinon and molinate.	
Pesticides	Ebro River Delta	Pesticide levels in water, metal body burdens, and up to 12 different biochemical markers were monitored in gills and digestive glands of oysters transplanted from May to June in 2008 and 2009. Biochemical responses evidenced clear differences in oysters from 2008 to 2009. Oysters transplanted in 2009 showed their antioxidant defenses unaffected from May to June and consequently increased levels of tissue damage measured as lipid peroxidation and DNA strand breaks and of mortality rates. Conversely oysters transplanted in 2008 increase their antioxidant defenses from May to June and had low levels of lipid peroxidation and DNA damage and low mortality rates. Some pesticides in water (bentazone and propanil) together with high temperatures and salinity levels were related with tissue damage in oyster transplanted in 2008, but the observed large differences between years indicate that abiotic factors alone could not explain the high mortalities observed in 2009	Ochoa et al. [98]

(continued)

Table 3 (continued)

Pollutants considered	Area/data source	Scope/remarks	References
Pharmaceuticals and pesticides	Water monitoring data collected in the Llobregat River basin MODELKEY research project	Risk assessment methodology: TU based on concentration addition mode for fish, *Daphnia*, and algae Survey was carried out along three campaigns in 7 sampling points, located in the main river and in one of its tributaries (Anoia River). In each sample, 29 commonly used pharmaceuticals, belonging to different therapeutic classes (analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs), lipid regulators, psychiatric drugs, antihistamines, antiulcer agents, antibiotics, and β-blockers), and 22 pesticides (herbicides and insecticides) have been determined Aggregated toxic units based on *Daphnia* and algae provided a good indication of the pollution pattern of the basin. Relative contribution of pesticides and pharmaceuticals to total toxic load was variable and highly site dependent, the latter group tending to increase its contribution in urban areas. Toxic units of the compounds identified in a sample fit a lognormal probability distribution. The parameters characterizing this distribution (mean and standard deviation) provide information tentatively interpreted as a measure of the toxic load and mixture complexity. Correlations of these parameters and 5 structural and functional biological descriptors related to benthic macroinvertebrates (diversity, biomass) and biofilm metrics (diatom quality, chlorophyll-*a* content, and photosynthetic capacity) are studied	Ginebreda et al.[99]

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Table 3 (continued)

Pollutants considered	Area/data source	Scope/remarks	References
Emerging and priority compounds	Water monitoring data collected in four Iberian Mediterranean River basins (Llobregat, Ebro, Júcar, Guadalquivir) by water authorities SCARCE-CONSOLIDER research project	Chemical and biological data gathered by four Spanish basin management authorities were examined with the following aims to (i) determine the chemicals most likely responsible for the environmental toxicological risk in the four Spanish basins and (ii) investigate the relationships between toxicological risk and biological status in these catchments. The toxicological risk of chemicals was evaluated using the toxic units (TU) concept. Analysis of the chemical data revealed high potential toxicological risk in the majority of sampling points. Metals were the main contributors to this risk. However, clear relationships between biological quality and chemical risk were found only in one river Data evaluation pointed to inadequacies in processing and monitoring (e.g., site coincidence for chemical and biological sampling)	López-Doval et al. [100]
Emerging and priority compounds	Water monitoring data collected in four Iberian Mediterranean River basins (Llobregat, Ebro, Júcar, Guadalquivir) by water authorities SCARCE-CONSOLIDER research project	The hazard of chemical compounds is prioritized according to their persistence, bioaccumulation, and toxicity properties by using self-organizing maps (SOM). An Integrated Risk Index of Chemical Aquatic Pollution (IRICAP), useful to assess the risk associated to the exposure of chemical mixtures present in river waters of Llobregat, Ebro, Júcar, and Guadalquivir. A SOM-based hazard index (HI) was estimated for ca. 200 organic micropollutants. IRICAP was calculated as the product of the HI by the concentration of each pollutant, and	Fàbrega et al. [101]

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Table 3 (continued)

Pollutants considered	Area/data source	Scope/remarks	References
		the results of all substances were aggregated According to the calculated HI, perfluoroalkyl substances, as well as specific illicit drugs and UV filters, were classified as most hazardous compounds. Xylazine had the highest contribution to the total IRICAP value in the different river basins, together with other pharmaceutical products such as loratadine and azaperol	
Emerging and priority compounds	Water monitoring data collected in four Iberian Mediterranean River basins (Llobregat, Ebro, Júcar, Guadalquivir) SCARCE-CONSOLIDER research project	The aims of the study were (a) to perform an environmental risk assessment for 200 organic micropollutants (pesticides, alkylphenols, pharmaceuticals, hormones, personal care products, perfluorinated compounds, and various industrial organic chemicals) monitored in four rivers of the Iberian Peninsula (Ebro, Llobregat, Júcar, and Guadalquivir Rivers) and (b) to prioritize them for each of the four river basins studied, taking into account their observed concentration levels together with their ecotoxicological potential. For this purpose, a prioritization *ranking index* (RI) associated with each compound was developed based on the measured concentrations of the chemical in each river and its ecotoxicological potential (EC50 values for algae, *Daphnia sp.*, and fish). Ten compounds were identified as most important for the studied rivers: pesticides chlorpyrifos, chlorfenvinphos, diazinon, dichlofenthion, prochloraz, ethion, carbofuran, and diuron and the industrial organic chemicals nonylphenol and octylphenol	Kuzmanovic et al. [13]

$$f_x = \frac{n_x}{N_{\text{total}}}\,(\%), \tag{5}$$

where n_x is the number of sites in the river basin falling in rank class x and N_{total} is the total number of sites per river. The sum of all the rank frequencies is equal to 100% as it covers all the sampling sites in the river basin. The compound's *ranking index* in the basin under study is defined by summing up the frequencies f_x multiplied by certain arbitrary weights w_x (Eq. 6):

$$\begin{aligned}\text{Ranking Index} =\; & \sum_{x=1}^{6} f_x \times w_x \\ =\; & (f_1 \times 1) + (f_2 \times 0.5) + (f_3 \times 0.25) + (f_4 \times 0.125) \\ & + (f_5 \times 0.0625) + (f_6 \times 0.0).\end{aligned} \tag{6}$$

The *ranking index* is scaled from 0 to 100, where 100 means that compound's log transformed TU is higher than 0 in all sites in sampled river and 0 that compound's log TU is not exceeding the value of −4 in any site. Following this approach, ca. 200 compounds belonging to different classes (pharmaceuticals, personal care products, industrial compounds, pesticides, perfluoroalkyl substances, and illicit drugs) were ranked according to their RI with respect to three organism indicators, i.e., algae, daphnids, and fish. Toxicity values were obtained from the literature and lacking values estimated from ECOSAR. The result of this exercise for the Llobregat River is reported in Table 4 [13] and Fig. 4. The most sensitive species as regards chemical risk seems to be *Daphnia* sp. followed by algae and fish. Nevertheless, algae seem more vulnerable to different classes of compounds. Even though pesticides are not the most dominant class in concentration, they dominate in terms of risk. Of key relevance are chlorpyrifos, diuron, diazinon, carbofuran, and azinphos-ethyl (RI $\geq$ 5), the former two being already included in Directive 2013/39/UE priority list. This is also the case of octylphenol, nonylphenol, and its transformation products (NP1EO, NP2EO, NP1EC) resulting from the degradation of polyethoxylated alkylphenols and also included as priority substances in the aforesaid directive. However, it is worth mentioning that all the classes considered are present in different extent in this basin-specific risk list.

Table 4 Compounds with risk index (RI) equal or higher than 1 in the water phase respect algae, *Daphnia* sp., and fish in the Llobregat River in 2010 and 2011 (compounds with RI exceeding 10% are highlighted in bold letters; compounds underlined are designated as priority substances under Directive 2013/39/UE) (adapted from [13])

Algae				***Daphnia*** **sp.**				Fish			
2010	RI%	2011	RI%	2010	RI%	2011	RI%	2010	RI%	2011	RI%
Diuron	**13**	Caffeine	7	**Chlorpyrifos**	**25**	**Chlorpyrifos**	**26**	**Chlorpyrifos**	**13**	Chlorpyrifos	9
Caffeine	7	Sertraline	6	**Diazinon**	**13**	**Diazinon**	**12**	NP1EC[a]	7	NP1EC[a]	1
Triclosan	5	Diuron	5	**Carbofuran**	**12**	NP1EC[a]	4	Nonylphenol	6		
Isoproturon	4	Terbutryn	3	**Octylphenol**	**12**	Octylphenol	4	NP2EO[a]	5		
Losartan	3	Triclosan	3	Azinphos-ethyl	9	Ethion	4	Malathion	4		
Nonylphenol	2	Simazine	2	Nonylphenol	6	NP1EC[a]	4	Gemfibrozil	4		
NP1EC[a]	2	Tolyltriazole	2	NP1EC[a]	6	Diuron	3	Bisphenol A	2		
Tolyltriazole	2	Benzotriazole	1	NP2EO[a]	5	Nonylphenol	2	L-PFOS	2		
NP2EO[a]	1	NP1EC[a]	1	Malathion	4	Dimethoate	1	Sulfamethoxazole	1		
Terbutryn	1	Nonylphenol	1	Chlorfenvinphos	3	Chlorfenvinphos	1				
Erythromycin	1	Isoproturon	1	Methiocarb	2	Tolyltriazole	1				
Clarithromycin	1	Atrazine	1	Azinphos-methyl	2						
Bisphenol A	1			Fenitrothion	1						
Prochloraz	1			Sertraline	1						
Sertraline	1			Venlafaxine	1						
Losartan	1			b							
Venlafaxine	1										
Valsartan	1										
L-PFOS	1										
Lorazepam	1										

[a]Degradation products of polyethoxylated nonylphenol

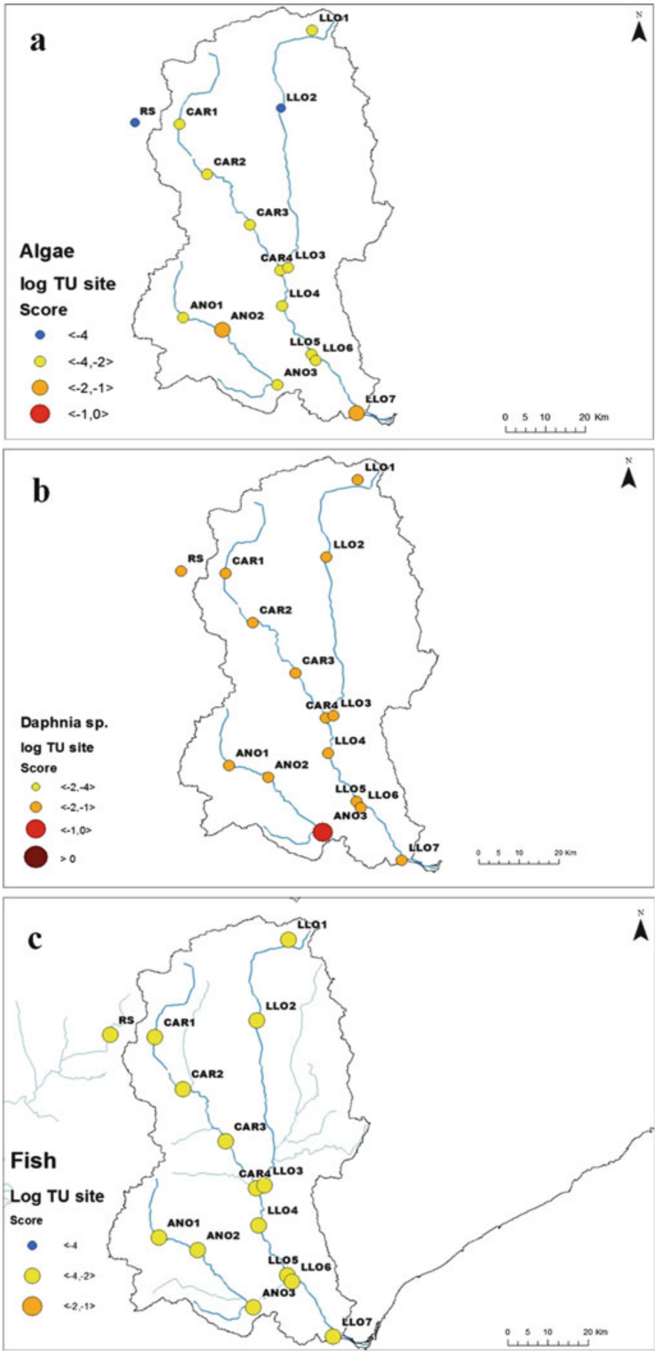

Fig. 4 Risk assessment associated to organic micropollutants in the Llobregat River basin expressed in log TU for different trophic levels. (**a**) *Daphnia*, (**b**) fish, and (**c**) algae (adapted from [13])

5 Concluding Remarks

Emerging contaminants are ubiquitous in basin rivers subjected to anthropogenic influence as it is the case of the Catalan Basin District and particularly that of the Llobregat River where most of the population is concentrated (specially in the lower part close to the Barcelona area). Owing to this fact, it is not strange that most of the studies concerning emerging contaminants were focused on this river. These studies carried out by either the water authorities or as part of research projects have shown the occurrence of compounds belonging to the most relevant families of emerging contaminants (pharmaceuticals, personal care products, industrial compounds, perfluoroalkyls, halogenated flame retardants, illicit drugs, pesticides, etc.), being their origin associated to both point (specifically WWTPs) or diffuse sources. In addition to parent compounds, the presence of transformation products resulting from either biotic or abiotic processes must be taken also into consideration on a more general perspective. Finally, the occurrence of emerging contaminants can be expressed in terms of environmental (ecotoxicity) risk which in turn can be consistently compared with the ecosystem status.

As a whole, it may be concluded that Catalan Rivers provide an interesting and illustrative example on how anthropogenic pressures translate into chemical pollution characterized by many families of non-regulated compounds and how this pollution may constitute a threat to the aquatic ecosystem.

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