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EFFECTS OF FLOW REGIME ON THE FISH COMMUNITIES OF THE LOWER EBRO RIVER

Núria Vila Martínez

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EFFECTS OF FLOW REGIME ON THE FISH COMMUNITIES OF THE LOWER EBRO RIVER

Núria Vila Martínez

Doctoral Thesis 2018

UNIVERSITAT
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Effects of flow regime on the fish communities of the lower Ebro River

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Núria Vila Martínez

DOCTORAL THESIS
2018

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Effects of flow regime on the fish communities of the lower Ebro River

DOCTORAL THESIS

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We STATE that the present study, entitled "EFFECTS OF FLOW REGIME ON THE FISH COMMUNITIES OF THE LOWER EBRO RIVER", presented by Núria Vila Martínez for the award of the degree of Doctor, has been carried out under our supervision at the Marine and Continental Waters Program (IRTA) and the Centre for Climate Change (Department of Geography, URV)

Vila-Seca, 5th of July of 2018

Doctoral thesis supervisors:

Dr. Javier Sigró Rodríguez

Dr. Carles Ibáñez Martí

Dr. Nuno Caiola

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I semblava que aquest moment no arribaria mai! Però aquí estem, i és l'hora de donar les gràcies:

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Contents

Abstract

Aquatic ecosystems are characterized by a strong coupling between physical forcing and ecological processes. In rivers, this physics-ecology interaction is driven by flow, one of the main factors determining biological integrity. The combination of high flow alterations due to dam regulation and an increasing presence of alien species is endangering native fish communities in the Iberian Peninsula. With a high number of endemisms, these communities are characterized by having developed complex mechanisms of adaptation to the natural changing flow of Mediterranean rivers, which the introduced species do not possess. The aim of this thesis is to explore the relationships between flow regime and fish communities in the lower Ebro River by analysing data from electrofishing campaigns collected during more than 10 years. We address this objective through three different approaches: On Chapter 1, the relationships between single species abundances and hydrological regime were evaluated while in Chapter 2 and 3 the objects of study were fish-based biological quality indices and size spectrum of fish comunity, respectively. To describe flow regime, we used a set of hydrological variables calculated from daily and hourly flow data series provided by the Ebro Basin Water authority (Confederation Hidrográfica del Ebro).

In the analysis of the responses of single species to changing flows, we found several significant relationships in both, seasonal and inter-annual variations. Alien species such as the Eastern mosquitofish (*Gambusia holbrooki*) and the topmouth gudgeon (*Pseudorasbora parva*), were negatively affected by high flows in annual and inter-annual variations, while the Pike-perch (*Sander lucioperca*) and the European perch (*Perca fluviatilis*) showed a negative response to high flows only in the annual cycle. On the other hand, several cyprinids were closely related with October and November flows (the bleak, *Alburnus alburnus*; the Iberian gudgeon, *Gobio lozanoi*; the goldfish, *Carassius auratus* and the Ebro barbel, *Luciobarbus graellsii*). In addition, we verified that some of the hydrological indices used underwent important changes with the construction of large dams in the river course. All these results lead us to propose the hydrological indices F_H3 (average magnitude of the flows above 3 times the median daily flow), M_H17 (25th percentile of the flow curve divided by median daily flow) and M_A13 (average November flow) to be studied as tools to stop the progress of invasive species in the area and protect the native ones.

The analysis of the relationship between interannual flow regimes and biological quality based on fish community, assessed through the Indices of Biotic Integrity in Catalan rivers

($IBICAT_{2010}$ and IBICAT2b) and the new European Fish Index (EFI+), showed that $IBICAT_{2010}$ was the most correlated with hydrological indices, followed by IBICAT2b and EFI+. Furthermore, high temporal and spatial scale dependences were observed in the patterns of correlation between biological quality indices and hydrological variables. On one hand, daily hydrological indices showed correlations with biological quality when they were computed using between 9 and 36 months of flow records (previous to the sampling date) whereas subdaily indices responded better to periods between 3 and 9 months of records. On the other hand, some sampling transects showed clearer relationships than others, even within the same water mass, which suggests an influence of the hydromorphological variability on the obtained biological quality scores.

Finally, the study of the entire fish community size-structure using Normalized Abundance Spectra (NAS) provided an approximation of the total fish abundance and food web capacity (through the parameter y-intercept) and an estimation of food web efficiency (through the slope of the linear regression). We detected significant relationships between NAS-related parameters and the hydrological variables describing diel flow oscillations and daily flow variability. Based on the results, we suggest that high flow variability conditions and, above all, high hydro-peaking conditions, caused a diminution of the total abundance of fish and a decrease of the proportion of small sized fish (i.e. lower y-intercept and flatter slopes of NAS, respectively). In addition, a significant interaction between hydro-peaking and the percentage of alien-predators specimens suggested that high hydro-peaking conditions benefit predation by facilitating predator-prey encounters. We concluded that the high proportion of alien fishes and the presence of a hydropower generation plant that operates by hydro-peaking are important factors determining fish size structure in the lower Ebro River.

Resum

Els ecosistemes aquàtics presenten una estreta connexió entre forces físiques i processos ecològics. Als rius, aquesta interacció física-ecologia és dirigida pel cabal, un dels factors principals que determinen la integritat biològica. La combinació de grans alteracions hidrològiques degudes a la regulació per preses i una creixent presència d'espècies al·lòctones, posa en perill les comunitats de peixos autòctons a la Península Ibèrica. Amb un gran nombre d'endemismes, aquestes comunitats es caracteritzen per haver desenvolupat mecanismes complexos d'adaptació al canviant règim hidrològic natural propi dels rius Mediterranis, que les espècies introduïdes no posseeixen. L'objectiu d'aquesta tesi és explorar les relacions entre el règim hidrològic i les comunitats de peixos del tram baix de l'Ebre per mitjà de l'anàlisi de dades de campanyes de pesca elèctrica realitzades durant més de 10 anys. Enfocàrem aquest objectiu a través de tres punts de vista diferents: Al capítol 1 s'avaluaren les relacions entre les abundàncies de cada espècie i el règim hidrològic, mentre que als capítols 2 i 3, l'objecte d'estudi foren índexs de qualitat biològica basats en peixos i l'espectre de mides de la comunitat íctica, respectivament. Per descriure el règim de cabals, férem servir un conjunt de variables hidrològiques calculades a partir de series diàries i horàries de cabals facilitades per la Confederació Hidrogràfica de l'Ebre (CHE).

En l'anàlisi de la resposta de cada espècie als canvis de cabal, trobàrem diverses relacions significatives en ambdues bases de dades, l'estacional i la inter-anual. Espècies al·lòctones com la gambúsia (*Gambusia holbrooki*) i la pseudoràsbora (*Pseudorasbora parva*), eren afectades negativament per cabals alts tan en el cicle anual com en els mostrejos inter-anuals, mentre que la sandra (*Sander lucioperca*) i la perca europea (*Perca fluviatilis*) mostraren una resposta negativa als cabals alts només durant el cicle anual. D'altra banda, diversos ciprínids resultaren estretament relacionats amb els cabals d'octubre i novembre (l'alburn, *Alburnus alburnus*; el gobi, *Gobio lozanoi*; el carpí, *Carassius auratus* i el barb de l'Ebre, *Luciobarbus graellsii*). A més a més, verificàrem que alguns dels índexs hidrològics utilitzats van sofrir importants canvis amb la construcció de les grans preses al curs del riu. Tots aquests resultats ens portaren a proposar els índexs hidrològics F_H3 (magnitud mitjana dels cabals superiors a 3 cops la mediana diària), M_H17 (percentil 25è de la corba de cabal dividit entre la mediana diària) i M_A13 (cabal mitjà de novembre) per ser estudiats com a eines per aturar l'avenç d'espècies invasores a l'àrea i protegir les natives.

L'anàlisi de les relacions entre els règims de cabals del període 2006-2016 i la qualitat biològica basada en la comunitat de peixos, determinada a través d'Índexs d'Integritat Biòtica pels rius catalans (IBICAT$_{2010}$ i IBICAT2b) i l'*European Fish Index* (EFI+), mostrà que IBICAT$_{2010}$ fou el més correlacionat amb els índexs hidrològics, seguit de l'IBICAT2b i l'EFI+. A més, una elevada dependència espacial i temporal fou observada en els patrons de correlació entre els índexs de qualitat biològica i les variables hidrològiques. Per una banda, els índexs hidrològics diaris van mostrar correlacions amb la qualitat biològica quan es van calcular en base a series de dades d'entre 9 i 36 mesos anteriors al mostreig, mentre que els índexs subdiaris respongueren millor quan es calcularen amb períodes d'entre 3 i 9 mesos. D'altra banda, alguns trams mostrejats presentaren relacions més clares amb el cabal que d'altres, fins i tot dins la mateixa massa d'aigua, cosa que suggereix la influència de l'hidromorfologia pròpia de cada tram sobre les puntuacions de qualitat biològica obtingudes.

Finalment, l'estudi de l'estructura de mides de la comunitat de peixos utilitzant l'Espectre de Mides Normalitzat (NAS, de les sigles en anglès), proporcionà una aproximació del total de l'abundància de peixos i la capacitat de la xarxa tròfica (a través del paràmetre intersecció amb l'eix y) i una estimació de l'eficiència de la xarxa tròfica (amb el pendent de la recta de regressió). Detectàrem relacions significatives entre els paràmetres derivats dels NAS i les variables hidrològiques descriptores d'oscil·lacions subdiàries de cabal i variabilitat diària. Basant-nos en aquests resultats, suggerim que les condicions d'alta variabilitat de cabal i, sobretot, la presència d'*hydropeaking,* causaren una disminució de l'abundància total de peixos i una disminució de la proporció de peixos petits (o sigui, menor intersecció amb l'eix y i pendents de NAS menys acusades, respectivament). També observàrem una interacció significativa entre l'*hydropeaking* i el percentatge de depredadors, suggerint que l'elevat *hydropeaking* beneficia la depredació facilitant els encontres entre depredadors i preses. Concloguérem que l'alt percentatge de peixos introduïts i la presència d'una planta de generació d'energia hidroelèctrica són els principals factors que determinen l'estructura de mides del tram baix de l'Ebre.

General introduction

A strong coupling between physical forcing and ecological patterns is found in aquatic ecosystems, where many biological processes are shaped and controlled by hydrological factors. This interaction in marine environments has led to the formulation of important oceanography theories such as the Main Sequence of phytoplankton life forms by Margalef (1978) or the Gran-Sverdrup Effect that explains primary production blooms in the open ocean (Gran, 1931; Sverdrup, 1953). In river ecosystems the physics-biology interaction is driven by flow, which is one of the main factors determining biological integrity (Fig. 1).

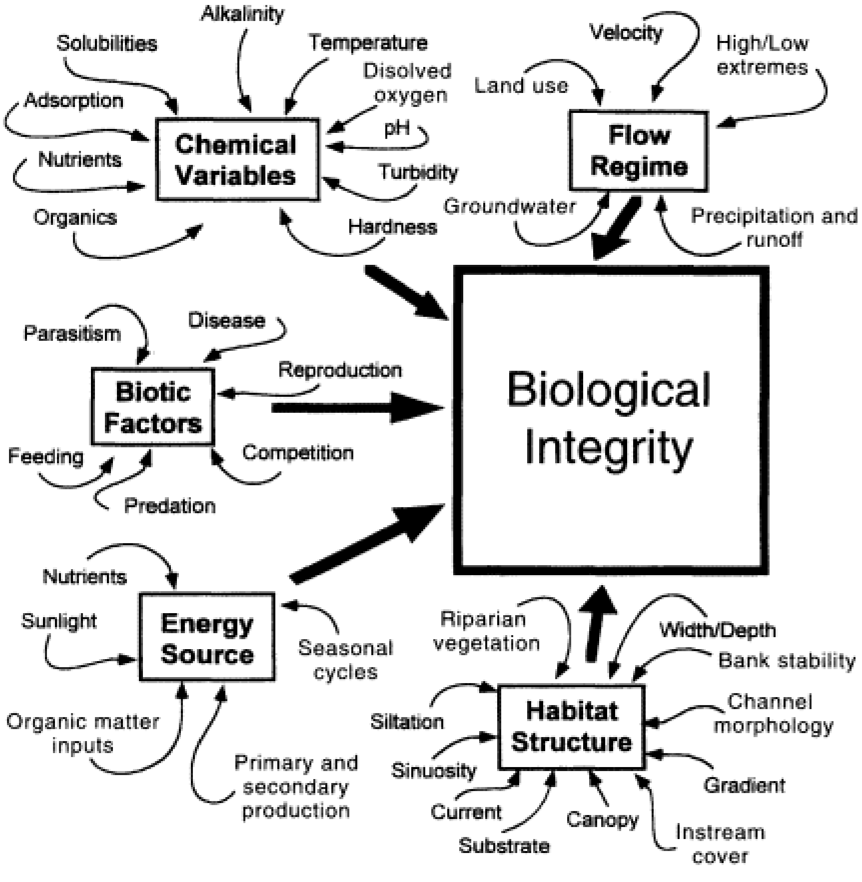

Fig. 1 The five principal factors that influence and determine the integrity of surface waters. (From: Schulze, 1999)

Poff et al. (1997) in their assay "*The Natural flow regime*" discussed the components of flow and the direct and indirect mechanisms through which it influences the characteristics and dynamics of river biological communities. Since then, aquatic ecologists and hydrologists have made great efforts to unravel the complex network of relationships between flow regime, biota and a whole range of other interacting environmental variables. Have been broadly analysed, for instance, the interconnections of flow with riparian vegetation (Merritt et al., 2010; Stromberg et al., 2007), macroinvertebrates (Theodoropoulos et al., 2017), fish (Caiola et al., 2014; Bailly et al., 2008; LeRoy Poff and Allan, 1995), macrophyte communities (Ibáñez et al., 2012a, 2012b), sediment transport and geomorphology (Rovira et al., 2015; Tena et al., 2012), and nutrient dynamics (von Schiller et al., 2011), among many others (Poff and Zimmerman, 2010). In this thesis, we investigated the effects of flow regime on fish communities in the lower part of the Ebro River, affected by multiple pressures and impacts of anthropogenic origin.

The use of Fish as bioindicators

The concept of ecological integrity was defined by Karr and Dudley (1981) 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". Therefore, the evaluation of the ecological integrity of an ecosystem, necessarily involves measuring the good state of its biotic communities. Fishes gather a series of characteristics that make them excellent bioindicators. The fact that they live permanently in water makes them integrate the possible chemical, physical and biological disturbances that occur in water bodies. Furthermore, the high longevity and broad distribution of some species enables the detection of disturbances over large temporal and spatial scales. Moreover, they are relatively easy to identify and sample, making their study more affordable than that of other biological groups (Jørgensen et al., 2016). Based on this, Karr (1981) proposed the first biological index: Index of Biotic Integrity, calculated from several fish-assemblage metrics. The IBI provides a numerical score for each sampling point that represents its ecological integrity compared with that of other areas less affected by anthropogenic disturbances (reference or least disturbed conditions) (Benejam et al., 2015).

Many variations of the fish based IBI were developed and applied to environmental quality assessment all around the world (Angermeier et al., 2000; Bozzetti and Schulz, 2004; Marzin et al., 2012) and was adapted to other groups of organisms such as macroinvertebrates (Alba-Tercedor et al., 2002; Archaimbault et al., 2010), macrophytes (Lacoul and Freedman, 2006; Riis et al., 2000) and diatoms (Besse-Lototskaya et al., 2011; Fore and Grafe, 2002). With the implementation of the Water Framework Directive (European Community, 2000), all the member states of the European Union are obliged to monitor the ecological status of water bodies in their countries through the use of multiple biological quality elements (BQE). But not all ecoregions possess properly regulated bioindicators to apply the Directive and for that, the literature and research projects related to the development of new indexes have increased considerably in the last two decades. The Iberian Peninsula is one of the ecoregions where the application of fish-based indexes is being very complex. A few native species, poor knowledge of their ecological requirements, many exotic species and a high number of endemisms with a wide range of tolerance to environmental variations hamper the applicability of fish biotic indices (Ferreira et al., 2007). And for this reason they are the object of constant studies and improvement efforts in the study area (D. Almeida et al., 2017; Benejam et al., 2008; García-Berthou et al., 2015). Among these efforts, it is worth mentioning the attempt to include size related variables in the bioassessment tools, since this is required by the Water Framework Directive (Murphy et al., 2013; Reyjol et al., 2014).

Community size structure

Size structures of aquatic communities are shaped by biotic interactions and environmental factors, and may therefore reflect changes occurred on the surrounding conditions, whether of anthropogenic or natural origin (Murry and Farrell, 2014; Sprules and Barth, 2016). But, what exactly do we mean when we talk about size structure? This is a concept originated with Elton (1927) observations in the beginning of trophic ecology, when he described an inverse relationship between the size of organisms and their number. Three decades later, by developing this premise, aquatic ecologists began to discuss what would eventually become the size spectrum theory that states that in aquatic ecosystems, the sum of biomass is approximately constant across equal logarithmic intervals of body size from the smallest to the largest organism (Sprules and Barth, 2016). From there, many ways to mathematically represent size structure in order to obtain synthetic variables has been developed. One of the most used is the Normalized Abundance Spectra (NAS) that can be adjusted to a linear model and provide information about food-web capacity (through the y-intercept of the spectrum) and ecological efficiency of the communities (through the slope of the spectrum) (Daan et al., 2005; Mehner et al., 2018; Rice and Gislason, 1996).

The sensitivity of size structure to external perturbations is a topic of growing interest among ecologists and there are several cases of success that demonstrate its usefulness for environmental management. The most consolidated example may be its use as a monitoring tool for marine communities that are subject to fishing exploitation (Bianchi, 2000; Rice and Gislason, 1996). Fish communities subjected to high fishing pressures tend to reflect the removal of large fishes and probably a diminution of predation on small fishes through steeper abundance spectrum slopes and the overall reduction of individual abundance through lower y-intercepts (Blanchard et al., 2005; Daan et al., 2005). The importance of these methods for the management of fishing exploitation is such that the European Union has included some of them in its Marine Strategy Framework Directive (European Union, 2008). Also for ecological quality assessment in freshwater ecosystems, a size-structure based approach could be very useful in areas where low species diversity limits the effectiveness of fish biotic indices based on community metrics, as is the case of the Iberian Peninsula. Murphy et al. (2013), for instance, analysed the response of several size related metrics of one fish (*Squalius laietanus*) to human disturbances and they obtained interesting results for species-specific applications and management. In the third chapter of this thesis, the utility of Normalized Abundance Spectra to detect anthropogenic perturbations in the study area is evaluated.

Study context: The lower Ebro River

The Ebro River crosses the Iberian Peninsula in the North West - South East direction flowing from the Cantabrian Range to the Mediterranean Sea, where it forms a Delta (Fig. 2). Its catchment has a total drainage area of about 85.550 km^2 and 12000 km of fluvial network. Is bounded by the Pyrenees in the north and the Iberian Range in the south (Herrero et al., 2018). The catchment is very heterogeneous in terms of its geology, climate and land uses, and presents a marked variability in water discharge. Throughout its course we can find several geological terrains that include sedimentary and metamorphic Paleozoic in the Iberian and Pyrenees mountains, igneous substrate in these two areas and in Priorat Massif, sedimentary Mesozoic substrate in the Iberian Chain and sedimentary Tertiary terrains in the Pre-Pyrenees and the Ebro Valley (Guillen and Palanques, 1992). Most of the basin presents a continental climate wit mountain climate at North (The Pyrenees) and Mediterranean climate at the lower area and near the coast (Ibáñez et al., 2008). Land uses have undergone changes over time, but nowadays irrigated cropland and pasture with fragmented vegetation patches dominate the landscape of the Ebro Basin (Xing et al., 2014). Like all Mediterranean rivers, it is subject to high variations in both intra-annual and between years flows. For example, the mean annual flow in Tortosa for the period 1981-2015 was 294.3 $m^3 \cdot s^{-1}$, with a maximum of 574.5 $m^3 \cdot s^{-1}$ (1988) and a minimum of 123.8 $m^3 \cdot s^{-1}$ (2012) and an absolute maximum of 3760 $m^3 \cdot s^{-1}$ during the 1982 flood (Confederación Hidrográfica del Ebro, 2009). Summer flows in dry years can reach very low values in River mouth such as for example the 70 $m^3 \cdot s^{-1}$ registered in Tortosa in 2012.

But the Ebro has been dramatically modified by human activity (Fig. 2), and its hydrology, together with all its associated processes, changed drastically with the construction of large dams during the sixties. There is a total of 125 reservoirs bigger than 1 hm^3 throughout the catchment, but the most important complex of reservoirs is located in the lower part and is

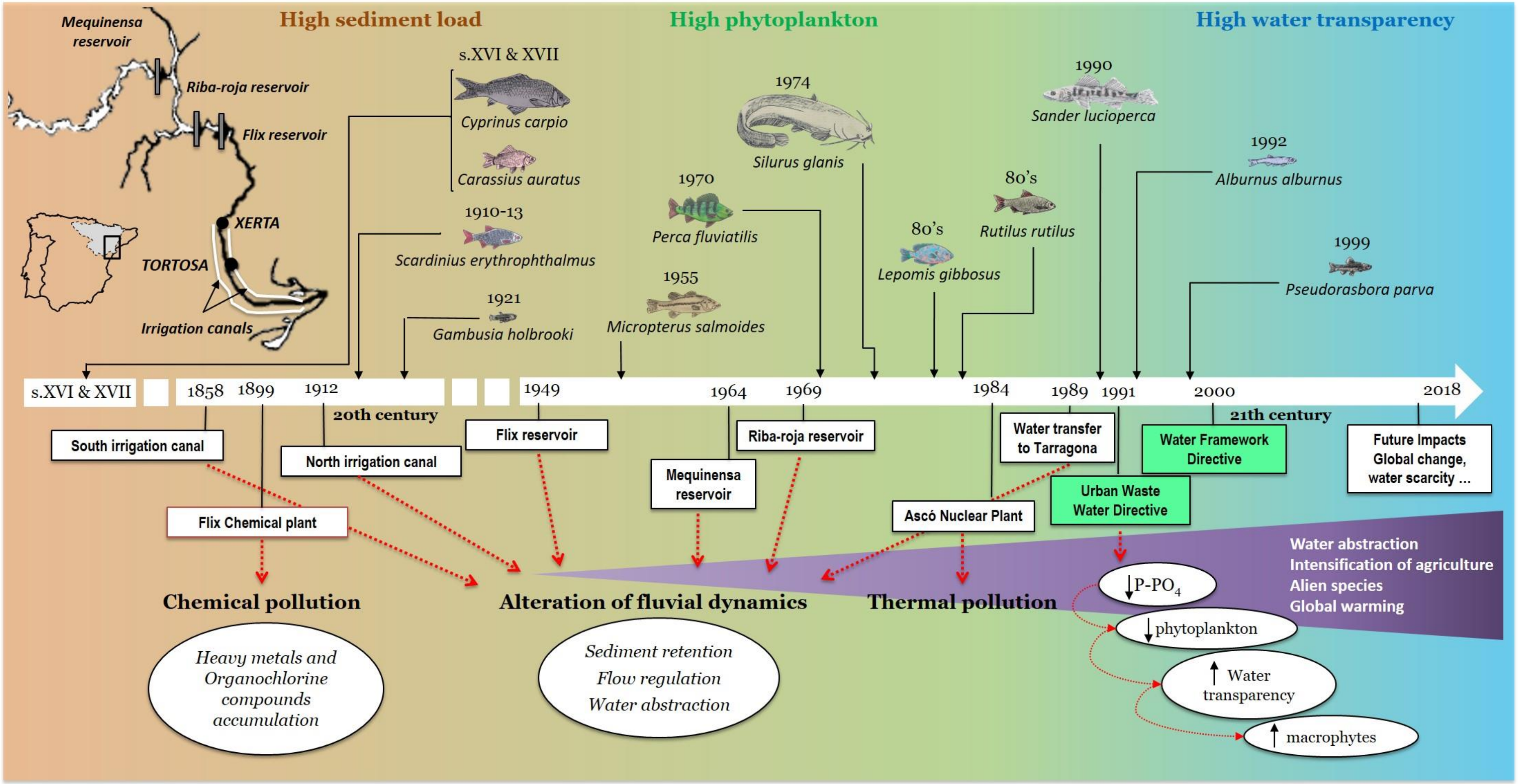

Fig. 2 Chronology of the main human impacts on the Lower Ebro River and dates of introduction of alien species. Adapted from Bentio (2016) and Cid (2010).

constituted by Mequinensa (operational from 1966 and with a capacity of 1534 hm^3), Riba-Roja (1969; 207 hm^3) and Flix (1948; 11 hm^3) (Batalla et al., 2008). They were built basically for hydroelectric production, control of floods and agricultural irrigation purposes. It is estimated that the water flow below Flix has been reduced by 25-30% because of regulation, and that 99% of the sediments that should naturally been transported by the river are retained in these reservoirs creating a downstream severe sediment deficit (Batalla et al., 2004; Ibàñez et al., 1996). As a result, the river channel morphodynamics is changing, and the Ebro Delta has ceased to grow, becoming now a more vulnerable system to the erosive processes of a storm wave-dominated coast (Benito et al., 2016; Genua-Olmedo et al., 2016; Rovira et al., 2015). Besides impeding the sediment transport, dam regulation has other direct and indirect effects over downstream ecosystems. The flow regime resulting from regulation involves the smoothing of flow peaks and the storage of autumn and spring floods, and, sometimes, the maintenance of higher summer flows than what would be expected in natural conditions (Batalla et al., 2004). These alterations can affect native fish by desynchronizing their reproductive cycles (Alexandre et al., 2015b, 2015a), by favoring the proliferation of invaders (Caiola et al., 2014) or altering the availability of prey organisms (Magalhães, 1993). Also the presence of a hydroelectric generation power plant in Flix that operates by hydro-peaking represents an important source of flow alteration. The hydro-peaking is the procedure by which hydroelectricity is produced to cover the daily fluctuating demands of the energy market and it results on rapid peaking flows that can fluctuate once or twice within 24 hours. This water management, although part of a system of clean energy production, has adverse effects on fluvial ecosystems, since organisms are not adapted to such rapid pulsed discharges that cause changes in stream habitat conditions (R. Almeida et al., 2017; Schmutz et al., 2015)

Another important source of human disruption in the lower Ebro River is the introduction and establishment of several alien fish species. Nowadays, the introduced species dominate the fish community, and represent a serious threat to the native biodiversity either by direct competition, predation, habitat alteration, hybridization with native species or introduction of parasites and diseases (Leunda, 2010). The reasons for which these species were introduced are very diverse, ranging from the illegal intentional release for recreational fishing in the case of Wels catfish (*Silurus glanis*) to legal intentional introduction for pest control in the case of Eastern mosquitofish (*Gambusia holbrooki*) and accidental introductions from aquariums such as the topmouth gudgeon (*Pseudorasbora parva*) (Table 1) (Elvira, 1995; Elvira and Almodóvar, 2001).

Latin name	Origin	Year of detection	Introduction path	Trophic competition	Spatial competition	Parasites or diseases introduction	Predation	Habitat alteration	hibridization
Alburnus alburnus	Central Europe	1992	Sport fishing		▨			▨	▨
Carassius auratus	Asia	s. XVII	Aquaculture		▨		▨	▨	▨
Cyprinus carpio	Eurasia	s-. XVI-XVII	Aquaculture	▨	▨			▨	
Gambusia holbrooki	North America	1921	Biologic conrol	▨	▨		▨		
Lepomis gibbosus	North America	80's	Aquarium	▨	▨		▨	▨	
Micropterus salmoides	North America	1955	Sport fishing	▨	▨		▨		
Pseudorasbora parva	Asia	1999	Aquarium	▨	▨	▨	▨		
Perca fluviatilis	Eurasia	1970	Sport fishing	▨		▨	▨	▨	
Rutilus rutilus	Central Europe	80's	Sport fishing	▨	▨			▨	
Scardinius erythrophthalmus	Eurasia	1910-13	Sport fishing	▨	▨				
Silurus glanis	Central Europe	1974	Sport fishing	▨	▨		▨	▨	
Sander lucioperca	Europe	1990	Sport fishing	▨	▨	▨	▨	▨	

Table 1. Origin, year of detection, introduction path and impacts contrasted in the bibliography (Modified from: López et al., 2012 and Aparicio et al., 2016)

As mentioned above, the Ebro River is affected by a long list of disturbance factors of anthropogenic origin. In addition, we must add chemical pollutants, thermal regime alteration due to a nuclear plant effluent, changes in land use and in the medium-long term, the effects of climate change. In this context of multiple stressors, the monitoring and restoration of ecological quality becomes an unavoidable challenge for water scientists and managers. With this thesis, we expect to contribute to a better understanding of the effects of flow regime and other stressors on fish communities and move towards a better management of hydrologically altered freshwater ecosystems.

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Objectives

The main goal of this thesis is to explore the relationships between fish community and hydrological regime in the lower Ebro River. To achieve this main objective, we propose the following specific objectives:

1- Characterize the fish communities, their temporal dynamics and species-hydrology interactions during:

a) The last 10 years (2006-2016)
b) The annual cycle 2014-2015
(Chapter1)

2- Determine the importance of hydrology on biological integrity by using fish-based biological quality indicators *(Chapter2).*

3- Assess the effects of human-induced flow oscillations and other stressors on the size structure of fish communities *(Chapter 3)*.

Chapter 1. Fish communities of the lower Ebro River: links with hydrological indices and flow tools for alien species control

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Abstract

The combination of high flow alterations due to dam regulation and an increasing presence of alien species is seriously endangering the integrity of the native fish communities in the Iberian Peninsula. With a high number of endemisms, these communities are characterized by having developed complex mechanisms of adaptation to the natural changing flow of Mediterranean rivers, which the introduced species do not possess. We analysed the relationship between flow alteration and fish abundance in the lower Ebro River on both annual (2006-2016) and monthly (2014-2015) data bases. We collected fish abundance data through monthly electrofishing campaigns during an annual cycle (2014-2015) and yearly sampling campaigns during the period 2006-2016 (11 years). Then, we used Mixed Effects Models to analyse possible linkages between abundance data and hydrological indices calculated from flow series provided by the Ebro Water Authority (CHE). We found that several species were significantly related with flow indices, both regarding seasonal and inter-annual variations. Alien species such as the Eastern mosquitofish (*Gambusia holbrooki*) and the topmouth gudgeon (*Pseudorasbora parva*), were negatively affected by high flows both, in annual and inter-annual variations, while the Pike-perch (*Sander lucioperca*) and the European perch (*Perca fluviatilis*) showed a negative response to high flows only in the annual cycle. On the other hand, several cyprinids were closely related with October and November flows: the alien bleak (*Alburnus alburnus*) increased in years of high average October flows; the native Iberian gudgeon (*Gobio lozanoi*) was benefited by high average flows during October and November; the alien goldfish (*Carassius auratus*) was less abundant when maximum November flows were higher and the native Ebro barbel (*Luciobarbus graellsii*) decreased with high maximum November flows. In addition, we verified that some of these hydrological indices underwent important changes with the construction of large dams in the river course and therefore, are the result of hydrological alteration. All these results lead us to propose the hydrological indices F_H3 (average magnitude of the occurrences during which the flow remains above 3 times the median daily flow), M_H17 (25th percentile of the flow curve divided by median daily flow) and M_A13 (average November flow) to be studied as tools to stop the progress of invasive species in the area and protect the native ones.

Key words

Invasive fish, flow alteration, fish-hydrology interaction, Mediterranean Rivers, ecological restoration

Resumen

La alteración de caudales debida a la construcción de grandes presas junto a un aumento de la presencia de especies introducidas, pone en grave peligro la integridad de las comunidades nativas de peces de la Península Ibérica. Con un gran número de endemismos, estas comunidades se caracterizan por haber desarrollado complejos mecanismos de adaptación a la variabilidad natural del caudal de los ríos Mediterráneos, que las especies introducidas no poseen. Hemos analizado la interacción entre alteración hidrológica y abundancias de peces en el bajo Ebro en series de datos anuales y mensuales. Se muestreó la comunidad de peces mediante campañas mensuales de pesca eléctrica durante un ciclo anual (2014-2015) y campañas anuales entre los años 2006 y 2016 (11 años). A continuación se usaron Modelos de Efectos Mixtos para analizar posibles vinculaciones entre las abundancias y un set de índices hidrológicos calculados a partir de series de caudales facilitadas por la Confederación Hidrográfica del Ebro (CHE). Varias especies resultaron significativamente relacionadas con los descriptores del régimen hidrológico, tanto por lo que respecta al ciclo anual como los cambios inter-anuales. Especies introducidas como la gambusia (*Gambusia holbrooki*) y la pseudorasbora (*Pseudorasbora parva*) se vieron afectadas negativamente por caudales altos tanto en el ciclo anual como en la serie inter-anual, mientras que el lucioperca (*Sander lucioperca*) y la perca europea (*Perca fluviatilis*) mostraron una respuesta negativa a caudales elevados sólo en el ciclo anual. Por otro lado, varios ciprínidos mostraron una estrecha relación con los caudales de octubre y noviembre: el alburno (*Alburnus alburnus*), especie introducida, aumentó en años de alto caudal medio de octubre; el autóctono gobio (*Gobio lozanoi)* se vio beneficiado por altos caudales medios tanto en octubre como en noviembre; el carpín (*Carassius auratus*), también introducido, fue menos abundante en años de elevados caudales máximos de noviembre y el barbo del Ebro, autóctono (*Luciobarbus graellsii*), disminuyó con altos caudales máximos de noviembre. Además, comprobamos que algunos de los índices hidrológicos analizados sufrieron importantes cambios con la construcción de grandes presas en el curso del río y, por lo tanto, son resultado de la alteración hidrológica. Por todo ello proponemos el estudio de los índices hidrológicos F_H3 (media de caudales por encima del triple de la mediana del caudal diario), M_H17 (percentil 25 de la curva de caudales dividido entre la mediana anual de caudales diarios) y M_A13 (caudal medio de noviembre) como potenciales herramientas para frenar el avance de especies invasoras y proteger las nativas.

Palabras clave

Peces invasores, alteración del caudal, interacción peces-hidrología, ríos Mediterráneos, restauración ecológica.

Introduction

The problem of alien fish invasions affects freshwater ecosystems all around the word endangering the integrity of native communities (Cucherousset and Olden, 2011). In the rivers and lakes of the Iberian Peninsula, a large number of introductions have been described in recent decades, in addition to some historical ones, which occurred hundreds of years ago (Elvira and Almodóvar, 2001; Leunda, 2010). In this ecoregion, highly variable environmental conditions led to the appearance of a large number of endemisms since the native fish populations had to adapt, in a process that lasted thousands of years, to extreme droughts in summer followed by strong floods in autumn and spring (García-Berthou et al., 2015; Ribeiro and Leunda, 2012) developing complex biological strategies that involve a close interaction with environmental factors. There have been many authors who have studied these interactions trying to decipher the secrets of the equilibrium achieved by native freshwater Mediterranean fishes. All they agree that the flow regime has been one of the key pieces in this evolutionary process, influencing reproductive cycles (Alexandre et al., 2015b, 2015a) growth rates (Herrera and Fernández-Delgado, 1992), feeding behavior (Magalhães, 1993), migration timing (Humphries et al., 1999; Ordeix et al., 2011) and, in summary, defining the entire structure of the native fish communities (Bernardo et al., 2003; Ribeiro et al., 2013).

Invasive species together with a big alteration of natural flow regimes due to dam regulation and water abstraction, seriously endanger the conservation of the endemic species richness in the aquatic ecosystems of the Iberian Peninsula. In addition to damage native species separately, these two factors multiply their harmful effect when they occur together (Ilhéu et al., 2014; Merciai et al., 2017; Oliveira et al., 2018). Batalla et al. (2004) described in detail the effects that the construction of dams in the Ebro basin had on the natural hydrological regime reporting reductions in flood magnitude, reductions in daily flow variability and increases in summer flows among many others. Such alterations have been the focus of numerous proposals and management programs for ecological restoration, based on observations of riparian forest (Magdaleno and Fernandez, 2011), hydromorphology (Ollero, 2010), sediment dynamics (Rovira et al., 2015), fish migration (Ordeix et al. 2011), among others. But there are in the area very few studies proposing flow management as a tool to diminish the presence of invasive fish (Caiola et al., 2014) although it is a subject widely studied in other watersheds of the world (Arthington and Balcombe, 2011; Lamouroux and Olivier, 2015; Lasne et al., 2007).

The main objective of the present study is to identify flow-ecology interactions on the fish community of the lower Ebro River through the analysis of species abundances and their linkage with a set of hydrological variables. Furthermore, we check if dam construction led to significant changes in these hydrological variables. Lastly, we propose the hydrological metrics that should be analysed in order to be used as management tools for the control of invasive species.

Material and methods

Study area

The Ebro River is one of the largest rivers in the Iberian Peninsula in terms of water volume and drainage area. It runs in the North West - South East direction, from the Cantabrian Mountains to the Mediterranean Sea, where it flows forming a Delta (Fig.1a). As all the Mediterranean rivers, it presents a great variability of flows between seasons and between dry and wet hydrological years. On average, its mean annual flow for the period 1980-81/2014-15 was 294.3 $m^3 \cdot s^{-1}$, with a maximum of 574.5 $m^3 \cdot s^{-1}$ (1987-88) and a minimum of 123.8 $m^3 \cdot s^{-1}$ (2011-12). The Ebro River basin encompasses 347 rivers, with a total length of approximately 12000 km (Confederación Hidrográfica del Ebro, 2009).

Our study was conducted in the lower Ebro River from the Flix dam to the upper limit of the estuary in Tortosa. As shown in figure 1b, six sampling stations were selected along the total length of the stretch that is about 80 km. Stations from E01 to E05 were chosen according to an analysis of aerial photographs that ensured the coverage of all the morphological variability of the area (Caiola et al. 2014). Station E06 was added later to represent the altered hydrological conditions that occur in this transect caused by the presence of a hydroelectrical power plant (Fig.1c).

Descriptors of hydrological regime

Hydrological data was gathered from two gauging stations (A163 and A027) from the Ebro river water authority (CHE) and the record of Flix dam discharge (Fig.1b). The latter is available since 2007 onwards. We used mean daily flow data series from the mentioned gauging stations, available upon request on the public CHE hydrological information service (www.saihebro.com), to characterize the flow regime. We used a set of hydrological indices described on Olden and Poff (2003) as the most suitable to characterize streamflow regimes on perennial rivers with influence of snow and rain events such as the Ebro River. The indices, their units and a brief description are presented in Table 1.

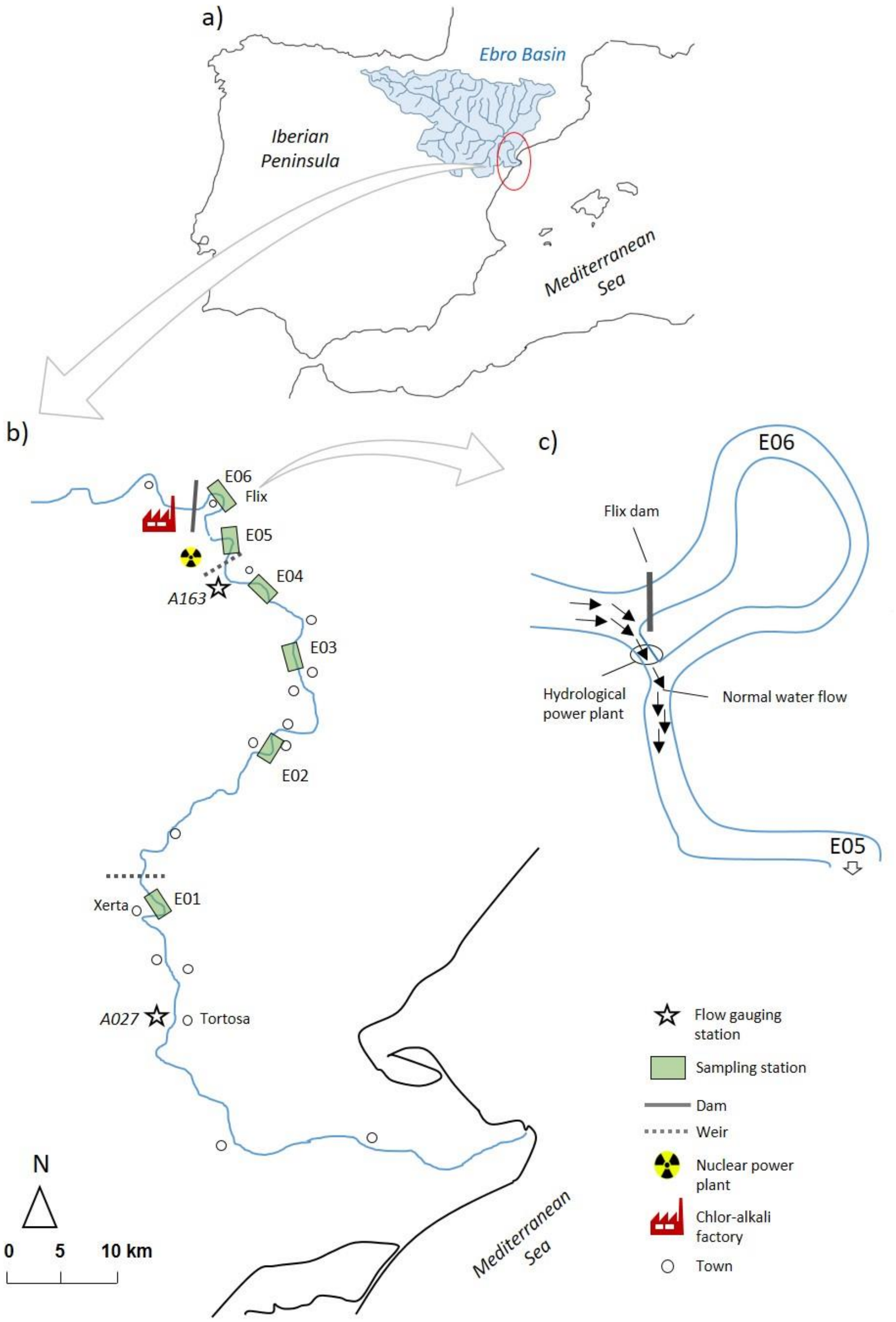


Figure 1. Study area. a) Geographical context. b) Location of river reaches and flow gauging stations. c) Diagram of the water flow deviation at station E06 by the presence of a hydroelectric power station.
Figura 1. Área de estudio. a) Contexto geográfico. b) Localización de los tramos de río y estaciones de medida del caudal. c) Diagrama de la desviación del agua en la estación E06 por la presencia de una central hidroeléctrica.

Code	**Units**	**Hydrological index**
Magnitude of flow events:		
M_A 3	-	Coefficient of variation in daily flows
M_A 44	-	Variability in annual flows divided by median annual flows, where variability is calculated as 90th-10th percentile
M_L 13	-	Coefficient of variation in minimum monthly flows
M_L 14	-	Mean of the lowest annual daily flow divided by median annual daily flow
M_H 17	-	25th percentile of the flow curve divided by median daily flow
M_H 20	m^3s^{-1}	Annual maximum flow
Frequency of flow events:		
F_L3	year^{-1}	Total number of low spells (threshold equal to 5% of mean daily flow)
F_L1	pulses	Number of annual occurrences during which the magnitude of flow remains below the 25th percentile
F_H3	m^3s^{-1}	Mean of flows during the occurrences in which the magnitude of flow remains above 3 times the median daily flow
F_H5	year^{-1}	Number of annual occurrences during which the magnitude of flow remains above the median daily flow
Duration of flow events:		
D_L1	m^3s^{-1}	Minimum annual daily flow
D_L13	-	Mean annual 30-day minimum divided by median flow
D_H12	-	Mean annual 7-day maximum divided by median flow
Timing of flow events:		
T_L1	-	Julian date of annual minimum
Rate of change in flow events:		
R_A8	year^{-1}	Number of negative and positive changes in water conditions from one day to the next
Other important indices in examining ecological response of fish assemblages		
M_A5	-	Skewness in daily flows: Mean daily flows divided by median daily flows
M_A12	m^3s^{-1}	Average October flow
M_A13	m^3s^{-1}	Average November flow
M_H1	m^3s^{-1}	Maximum October flow
M_H2	m^3s^{-1}	Maximum November flow

Table 1. Descriptors of hydrological regime adapted from Olden & Poof (2003). *Tabla 1. Descriptores del régimen hidrológico adaptados de Olden & Poof (2003).*

Flow data from Flix dam were assigned to sampling station E06; data from gauging station A163 were assigned to sampling stations E02 to E05, and flows measured on station A027 were assigned to sampling station E01. The indices were computed in two ways: 1) considering the whole hydrological year (from October to next September) for the assessment of inter-annual sampling campaigns performed between 2006 and 2016 and 2) considering only one month before sampling for the annual cycle campaign carried out during the hydrological year 2014-2015. In the second case, only half of the indices could be adapted to a period of one month. The rest was discarded because they only make sense if they are calculated for a whole hydrological year or because they presented no variability along the annual cycle. The indices used for annual cycle analysis were M_A3, M_A44, M_H17, M_H20, F_L1, F_H5, D_H12, R_A8 and M_A5.

To compare the values of these set of hydrological descriptors before and after the construction of large dams, we also computed the indices for hydrological years during the period 1923-24/1933-34 (before dam regulation) with mean daily flow data from gauging station A027 in Tortosa (Fig. 2).

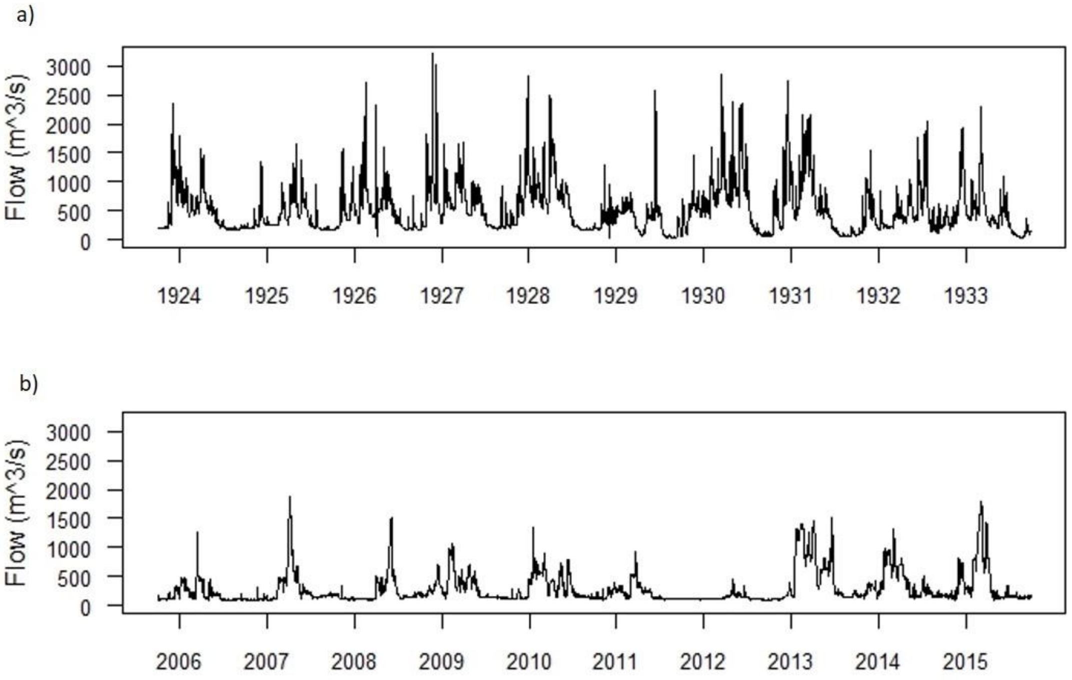


Figure 2. Hydrographs a) before dam construction (1923-24/1933-34) and b) after dam construction (2005-06/2015-16) in Tortosa. *Figura 2. Hidrogramas a) antes de la construcción de las grandes presas (1923-24/1933-34) y b) después (2005-06/2015-16) en Tortosa.*

Fishing campaigns

Two types of electrofishing campaigns were conducted to characterize the fish community of the lower Ebro. The first consisted in yearly samplings from 2006 to 2016 carried out between summer and early autumn (hereafter "inter-annual" data), and the second was performed between November 2014 and October 2015 and consisted in monthly samplings (hereafter "annual cycle"). In the annual cycle, the samplings corresponding to December, February and March were not carried out because the high flow conditions (above 500 $m^3 \cdot s^{-1}$) would have compromised the safety of the crew.

The same sampling design used by Caiola et al. (2014) was applied. To meet the European standards for electrofishing (CEN. European Committee for Standardization, 2003) that stablishes a sampling stretch length of approximately ten times the river width (150 - 200 m), 2 km long sampling stations were delimited. Within each sampling station 10 equidistant points located in riverbanks were fished from a boat. An electro-fishing gear (Model: EL63IIGI, HANS GRASSL GmbH, Schönau am Königssee, Germany) which generated 600 V and 10 A pulsed D.C. was used to perform a two-passes sampling, going from downstream to upstream. The fish were collected with dip nets of 7 mm of mesh size and 2.5 m long, for a distance of 10 m, covering an area of 25 m^2. The fish were kept in containers with river water while handled. Fish specimens were sorted, identified to species level, counted and weighted. Alien species were eliminated with an overdose of anesthetic MS-222 while native species were returned to the river after a short recovering period. The abundances were normalized dividing by fished area and time of fishing to be expressed as catches per unit effort (CPUE: individuals$\cdot ha^{-1}\cdot min^{-1}$) and biomass per unit effort (BPUE: $kg\cdot ha^{-1}\cdot min^{-1}$).

Data analysis

Common descriptors of biological communities were calculated. Species richness, Shannon diversity index and percentage of alien species were computed for inter-annual data and annual cycle using individuals abundances expressed as CPUEs (individuals$\cdot ha^{-1}\cdot min^{-1}$) and biomass abundances expressed as BPUEs ($kg\cdot ha^{-1}\cdot min^{-1}$). We checked for general tendencies in these descriptors during the study periods.

To explore possible relationships between hydrological regime and species abundances, we adjusted Linear Mixed Effects Models between each hydrological descriptor and species abundances. Mixed Effects Models are used to avoid the invalidation of some statistical tests derived from a lack of independence in data sets (Zuur et al., 2009). In our case, as we have several data for each river sampling station, the abundances within these data sets are likely to be more related to each other than to the abundances on different sampling stations. Linear regression models do not take this relatedness into account. The R package nlme (Pinheiro et al., 2017; R Core Team, 2016) was used for the analysis. We adjusted the models with the following response variables: species richness, Shannon diversity index (based on CPUE and BPUE), percentage of alien species (based on CPUE and BPUE), CPUE and $\log_{10}$ transformed CPUE per species, BPUE and $\log_{10}$ transformed BPUE per species. Only significant models with normally distributed residuals were considered for discussion.

We performed a comparison of hydrological descriptors before and after the construction of big dams on the study area. Tukey tests were computed to compare indices of the periods 1923-24/1933-34 (before dam regulation) and 2005-06/2015-16 (under dam regulation). In this way we wanted to establish which of the used indexes could be considered indicators of hydrological alteration due to dam regulation.

Results

Community composition

Annual cycle

Throughout the study period November 2014 – October 2015, 31186 fishes were accounted. They were classified into 18 species plus the category "undetermined mullet", which included individuals of the family Mugilidae belonging to the species *Mugil cephalus*, *Liza ramada*, *Liza saliens* (unlikely but possible), *Liza aurata* (unlikely but possible) and *Chelon labrosus.* Among all catches there were 6 native species plus the native group of undetermined mullets and 12 introduced species. From the list shown in Table 2, the native sand smelt (*Atherina boyeri*) was not found during the annual cycle.

Species richness remained between 16 and 19 (Fig. 3a) presenting the lowest values on April and May, months in which Eastern mosquitofish (*Gambusia holbrooki*) and largemouth black bass (*Micropterus salmoides*) were absent, together with European perch (*Perca fluviatilis*) on April and the sea bass (*Dicentrarchus labrax*) on May. The sea bass was also not caught in July and October, while in November were absent the mullets and the pike-perch (*Sander lucioperca*) and in June the largemouth black bass. The highest values were found in January, August and September, when all the species were caught except the sand smelt.

In regards to Shannon diversity index it varied between 0.9 and 1.9 for all the samplings (Fig. 3a). The highest value was found in August for the CPUEs diversity while in April for the BPUEs diversity. The percentage of introduced individuals and biomass remained very high during all the period; on average 78 % for CPUEs and 84 % for BPUEs. There was only one campaign when the percentage of alien individuals fell below fifty percent: on May 2015.

Family	Species	Code	Common name	Status	Total catches
Anguillidae	*Anguilla Anguilla*	aan	European eel	N	5800
Ciprinidae	*Alburnus alburnus*	aal	Bleak	A	42856
	Luciobarbus graellsii	lgr	Ebro barbel	N	335
	Carassius auratus	cau	Goldfish	A	379
	Cyprinus carpio	cca	Common carp	A	1185
	Gobio lozanoi	glo	Iberian gudgeon	N	598
	Rutilus rutilus	rru	Roach	A	3764
	Pseudorasbora parva	ppa	Topmouth gudgeon	A	3343
	Scardinius erythrophthalmus	ser	Rudd	A	707
	Squalius laietanus	sla	Catalan chub	N	2038
Siluridae	*Silurus glanis*	sgl	Wels catfish	A	611
Poecilidae	*Gambusia holbrooki*	gho	Eastern mosquitofish	A	11135
Atherinidae	*Atherina boyeri*	abo	Sand smelt	N	156
Bleniidae	*Salaria fluviatilis*	sfl	Freshwater blenny	N	1271
Centrarchidae	*Lepomis gibbosus*	lgi	Pumpkinseed	A	1410
	Micropterus salmoides	msa	Largemouth black bass	A	83
Percidae	*Perca fluviatilis*	pfl	European perch	A	253
	Sander lucioperca	slu	Pike-perch	A	914
Mugilidae	Undetermined Mullet	min	Mullet	N	655
Moronidae	*Dicentrarchus labrax*	dla	Seabass	N	27

Table 2. List of species found. Latin names, codes, common names, status refereed to the Ebro River (N=native; A=alien) and total catches. *Tabla 2. Lista de especies encontradas. Nombre científico, código, nombre común, estado referente al río Ebro (N=nativa, A= introducida) y total de capturas.*

In figure 4 we present the evolution of the abundances of each species in CPUEs and in BPUEs. The most abundant species in terms of individuals was the bleak (*Alburnus alburnus*), followed by the European eel (*Anguilla anguilla*) (Fig. 4b) whereas in terms of biomass were the common carp (*Cyprinus carpio*) and again, the bleak (Fig. 4c). The rarest specimens were of sea bass and largemouth black bass, while in biomass, the scarcest species were the Iberian gudgeon (*Gobio lozanoi*) and the largemouth black bass.

With regards to spatial distribution, the sea bass and the mullets were found almost exclusively in the first sampling station E01, downstream of Xerta weir. Only 7 of 392 mullets were caught in stations upstream Xerta. These are, together with the sand smelt, marine species tolerant to freshwater that sporadically visit the study area in search of feeding resources. On the contrary, largemouth perch and European perch showed preference for the station located most upstream since 96% of largemouth perch and 81% of European perch were fished in E06.

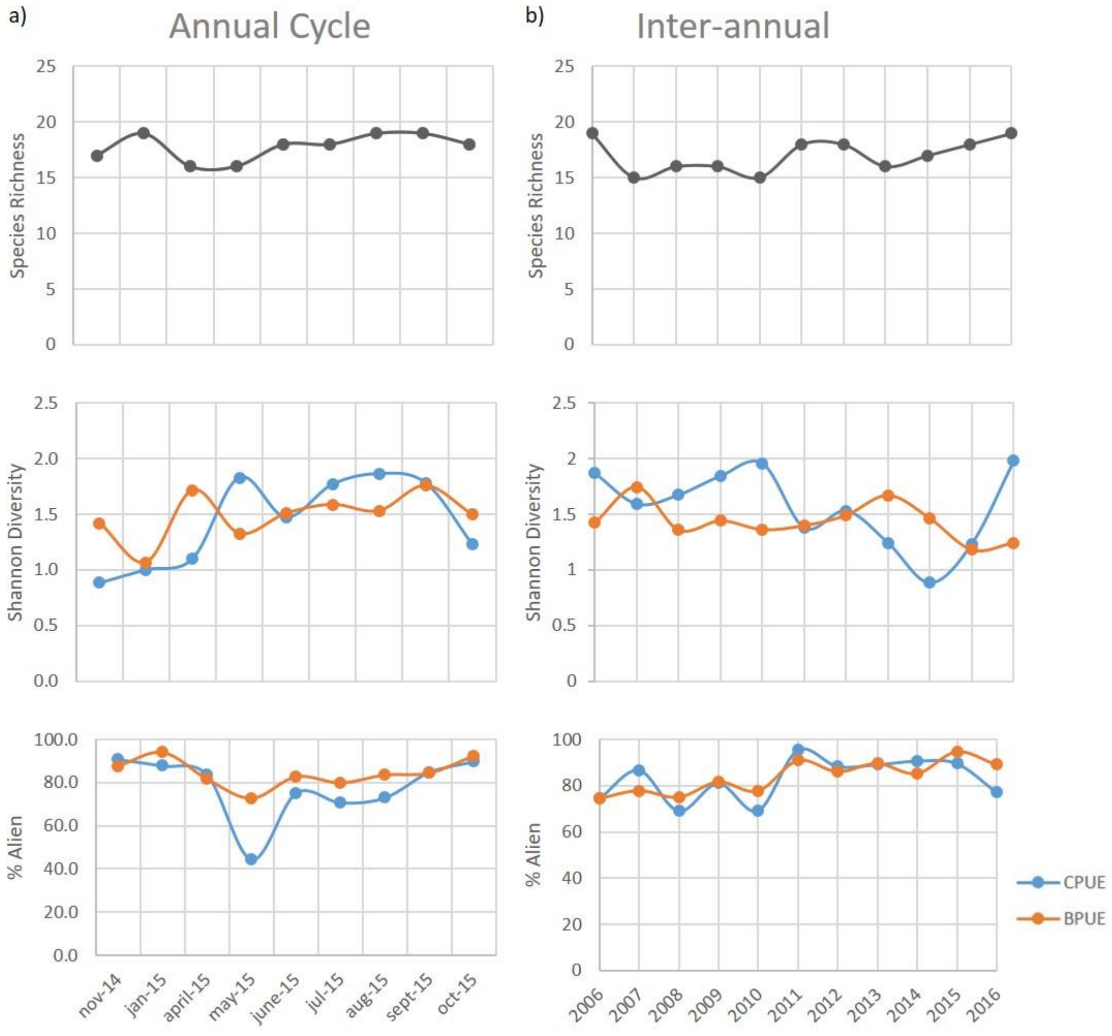


Figure 3. Community metrics a) per sampling month during an annual cycle and b) per sampling year during the period 2005-06/2015-16. *Figura 3. Métricas de comunidad a) por mes de muestreo durante un ciclo anual y b) por año de muestreo durante el periodo de estudio 2005-06/2015-16.*

Inter-annual samplings

During the inter-annual samplings a total of 46334 fishes were caught belonging to 7 native species and the native group “undetermined mullets” and 12 introduced species (Table 2). Richness oscillated between 15 and 19 (Fig. 3b). The European perch was fished from 2014 onwards suggesting its expansion in the lower Ebro coming from upstream big reservoirs, were is well stablished since its introduction for sport fishing purposes in the 70’s (Elvira and Almodóvar, 2001). The less frequent species was the sand smelt that only was fished twice; in

2006 and 2012. The other marine species also appeared irregularly: the sea bass (fished in 2006, 2009, 2011, 2012, 2014 and 2016) and the mullets (absents in 2013 and 2014). Moreover, other species were not found in some samplings campaigns: the rudd (*Scardinius erythrophthalmus*) was absent in 2007 and 2010; the Catalan chub (*Squalius laietanus*) was not fished between 2008 and 2010; the largemouth black bass was absent in 2009 and 2010; the Iberian gudgeon was absent in 2012 and the pike-perch was not found in 2014.

In regards to Shannon diversity index it varies between 0.9 and 2 for all the samplings. The index based on catches per unit effort presented more variability than the one calculated for biomass per unit effort (Fig. 3b). The percentage of introduced individuals and biomass remained very high in all the samplings; on average 83 % for CPUEs and 84 % for BPUEs. This value never went below 69% and it presented a slightly increasing tendency.

In figure 5 we present the evolution of the abundances of each species in CPUEs and in BPUEs for the inter-annual data set. The most abundant species in terms of individuals was the bleak followed by the Eastern mosquitofish (Fig. 5b), whereas in terms of biomass were the common carp and the Wels catfish (*Silurus glanis*) (Fig. 5c). All them were introduced species. The rarest specimens were of sea bass and largemouth black bass, while in biomass, the scarcest species were Iberian gudgeon and the European perch.

As observed in the annual cycle, the marine species mentioned above were found almost exclusively in the first sampling station E01, downstream of Xerta weir. Only 7 of 249 mullets were caught in the stations upstream Xerta. And again, European perch showed preference for the station located most upstream since 81% of its individuals were fished in E06.

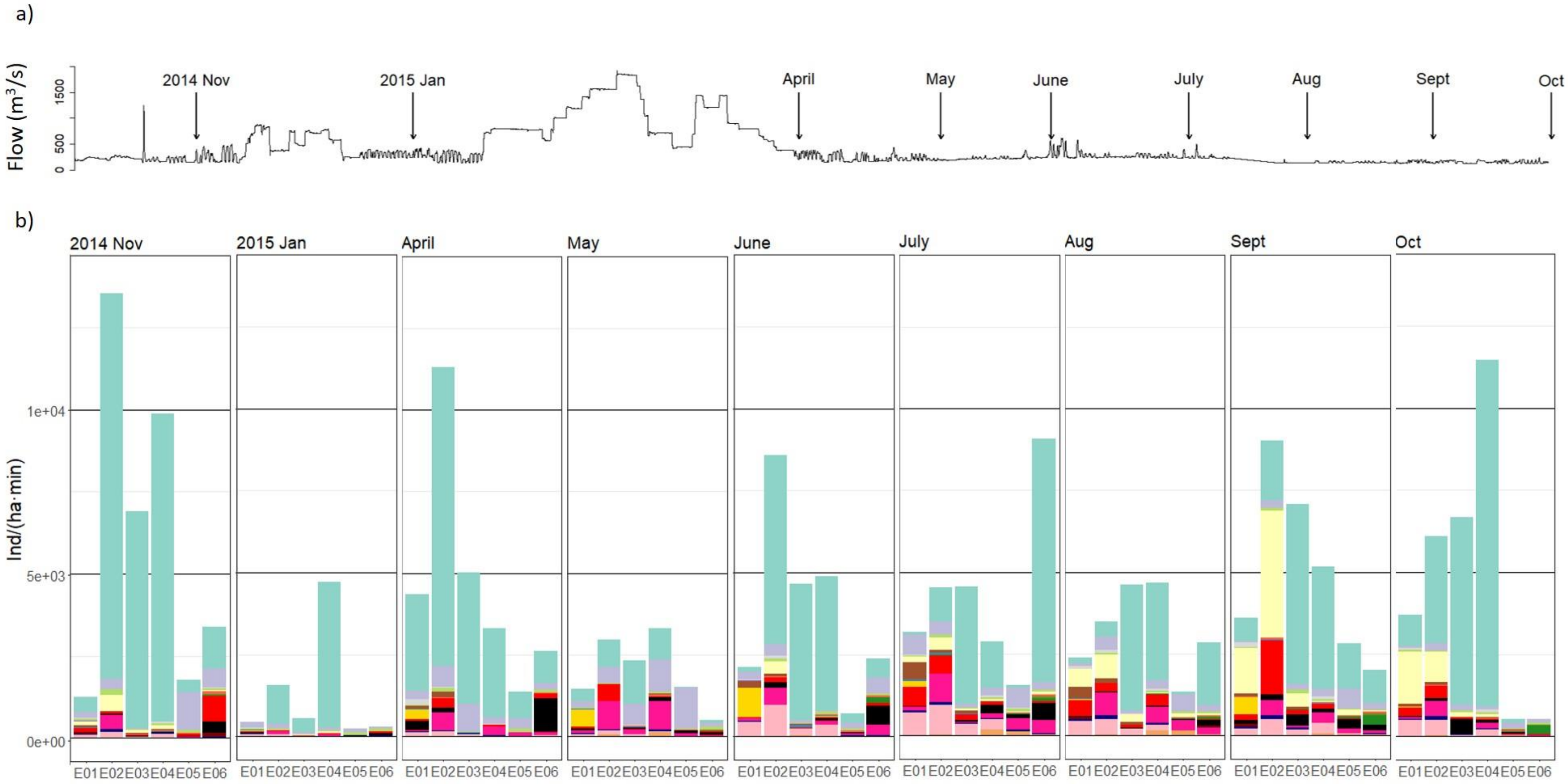
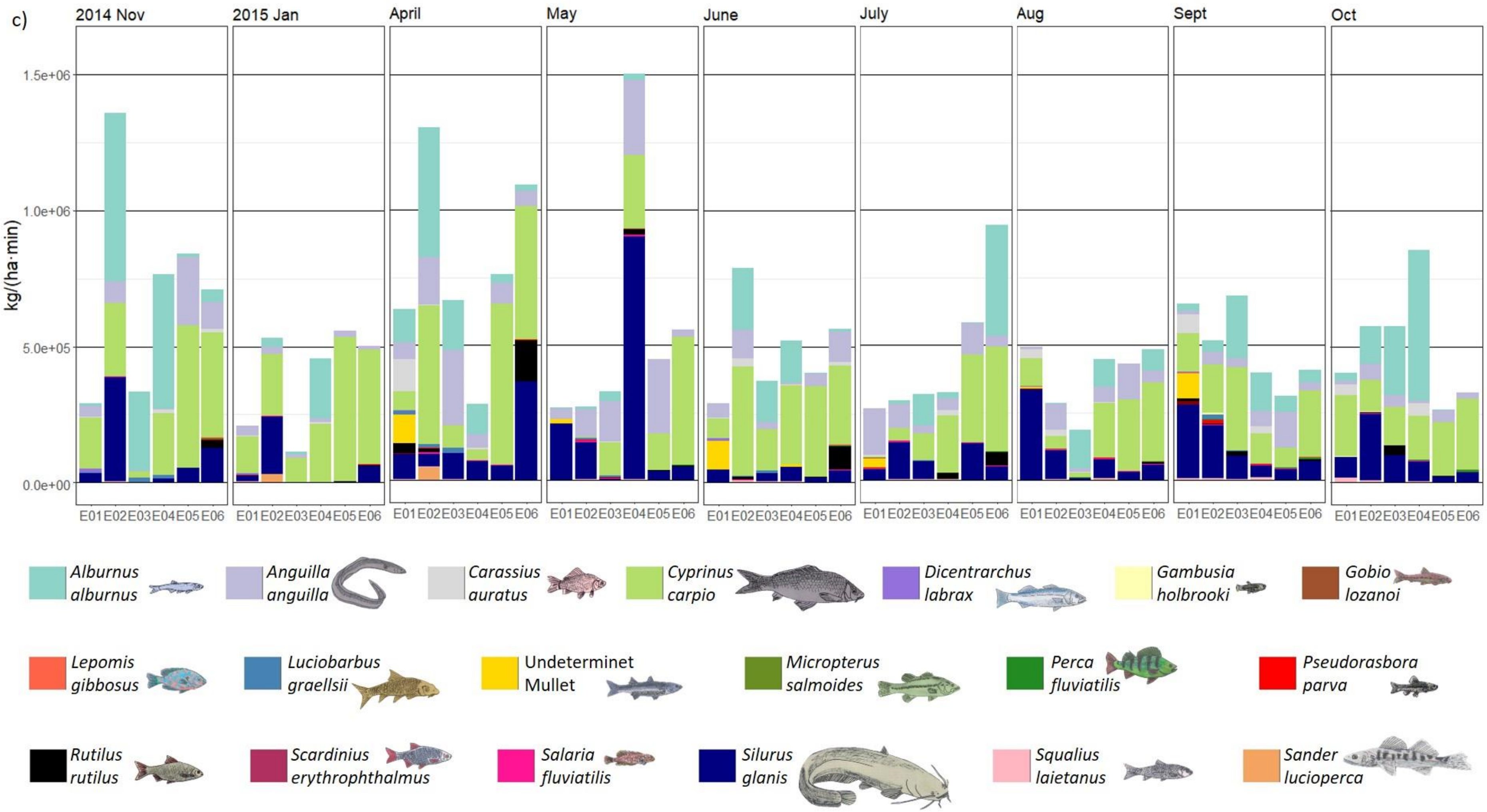


Figure 4. a) Hydrograph for the study period November 2014 - October 2015. Arrows indicate sampling dates. b) Temporal variation of individual abundances by river reach. c) Temporal variation of biomass abundances by river reach. *Figura 4. a) Hidrograma para el periodo de estudio Noviembre 2014 - Octubre 2015. Las flechas indican los días de muestreo. b) Variación temporal de las abundancias de individuos por tramo de río. c) Variación temporal de las abundancias de biomasa por tramo de río.*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Cyprinus carpio
Dicentrarchus labrax
Gambusia holbrooki
Gobio lozanoi
Lepomis gibbosus
Luciobarbus graellsii
Undeterminet Mullet
Micropterus salmoides
Perca fluviatilis
Pseudorasbora parva
Rutilus rutilus
Scardinius erythrophthalmus
Salaria fluviatilis
Silurus glanis
Squalius laietanus
Sander lucioperca

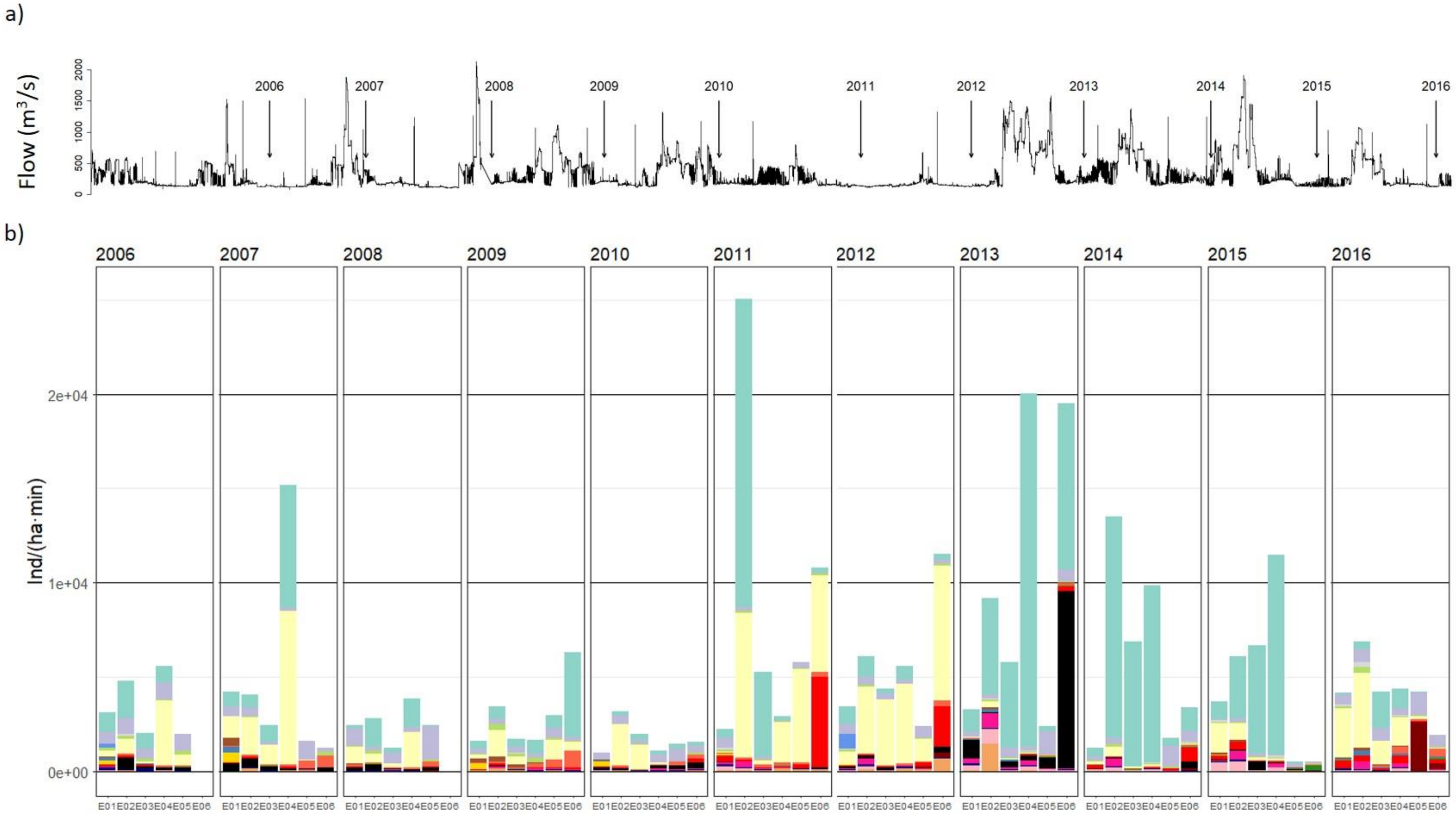
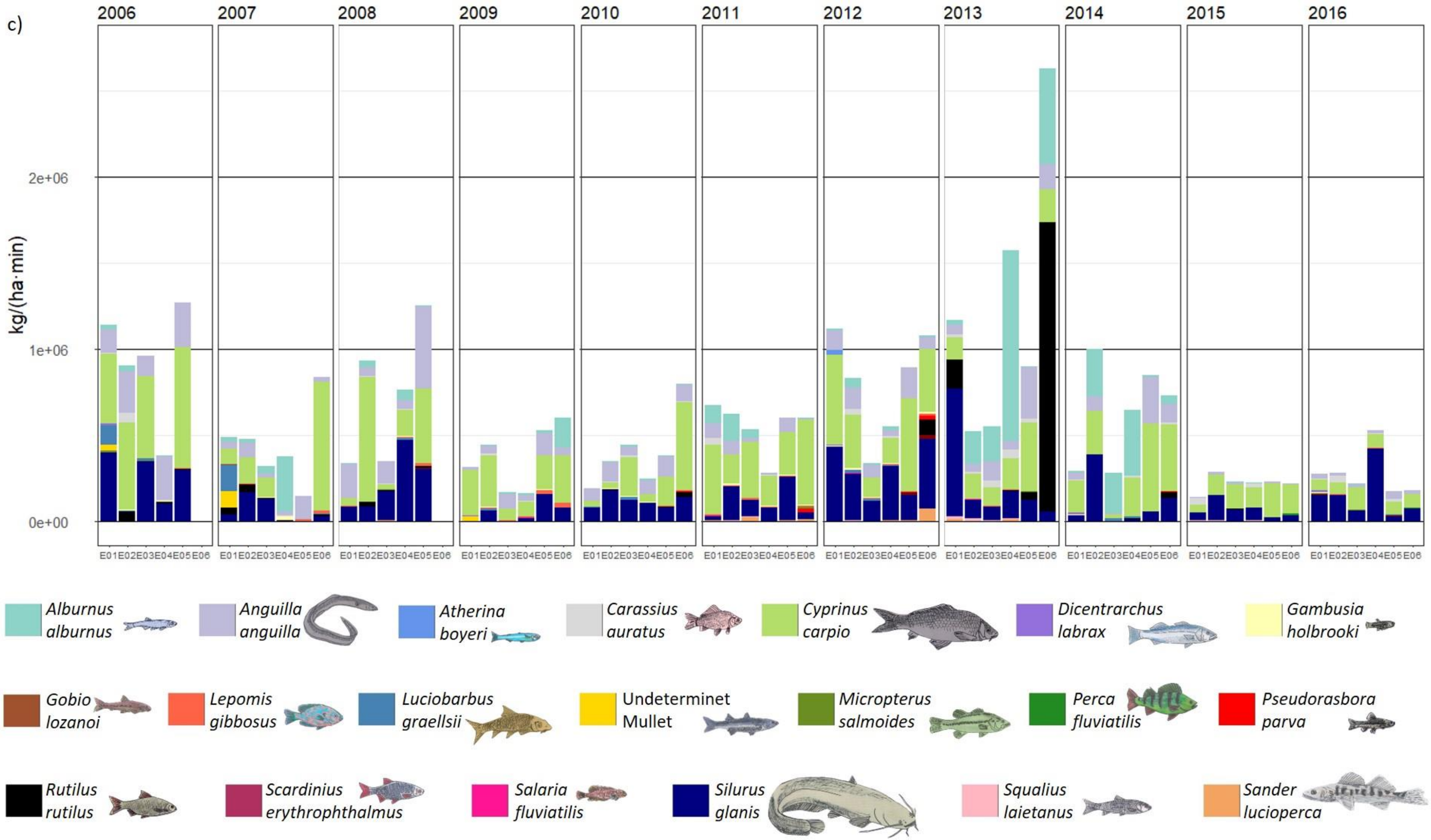


Figure 5. a) Hydrograph for the study period 2005-06/2015-16. Arrows indicate sampling dates. b) Temporal variation of individual abundances by river reach. c) Temporal variation of biomass abundances by river reach. *Figura 5. a) Hidrograma para el periodo de estudio 2005-06/2015-16. Las flechas indican los días de muestreo. b) Variación temporal de las abundancias de individuos por tramo de río. c) Variación temporal de las abundancias de biomasa por tramo de río.*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Atherina boyeri
Carassius auratus
Cyprinus carpio
Dicentrarchus labrax
Gambusia holbrooki
Gobio lozanoi
Lepomis gibbosus
Luciobarbus graellsii
Undeterminet Mullet
Micropterus salmoides
Perca fluviatilis
Pseudorasbora parva
Rutilus rutilus
Scardinius erythrophthalmus
Salaria fluviatilis
Silurus glanis
Squalius laietanus
Sander lucioperca

Influence of hydrological regime on species abundances

Annual Cycle

For the annual cycle data set we found that species richness was significant and positively related with the descriptor of high flow conditions M_H17 and negatively related with M_H20 and F_H3, calculated for one month before sampling. The Shannon Diversity index based on number of individuals also was found to be positively related with M_H17 and negatively related with D_H12 and F_H3. Shannon Diversity based on biomass was negatively related with F_H5.

Regarding the abundances of each species expressed in $\log_{10}$+1 transformed CPUEs, the European eel, the European perch, the roach (*Rutilus rutilus*), the rudd and the pike-perch provided significant models with some of the indices. With respect to biomass abundances, the species that gave up significant models were: Common carp (expressed in BPUEs), Eastern mosquitofish, European perch, topmouth gudgeon (*Pseudorasbora parva*) and the pike-perch ($\log_{10}$+1 transformed BPUEs) (Table 3). The hydrological indices that showed significant relationships with one or more species abundances were M_A3, M_H17, M_H20, F_H3, F_H5, D_H12 and R_A8 (Fig. 6).

Inter-annual samplings

For the inter-annual data set, species richness gave significant models when related with M_L13, M_H2 and F_L1. Shannon diversity index was significant and negatively related with M_H2 in its two calculation modalities (using CPUEs and BPUEs), the percentage of alien individuals appeared to be negatively related with M_H1 and the $\log_{10}$+1 transformed total biomass, negatively related with F_H5.

The number of species abundances found to be significantly related with hydrological indices yearly calculated was greater than for the annual cycle. The goldfish (*Carassius auratus*), the Eastern mosquitofish, the Iberian gudgeon, the topmouth gudgeon and the roach presented significant relations with hydrology expressed in both, CPUEs and BPUEs $\log_{10}$ +1 transformations. Moreover, the bleak, the pumpkinseed (*Lepomis gibbosus*) and the Ebro barbel were significant when expressed as $\log_{10}$ +1 CPUEs, and the European eel when expressed as $\log_{10}$ +1 BPUEs. (Table 4). The hydrological indices that showed significant relationships with one or more species abundances were M_A3, M_L13, M_L14, M_H17, M_H20, F_L1, F_H3, F_H5, D_L1, M_A12, M_A13, M_H1 and M_H2 (Fig. 7).

Response variable	Predictor	Estimate	p_value	R^2
Richness	M_H17	7.62	<0.001	0.46
	M_H20	$-3.78\cdot10^{-3}$	<0.01	0.41
	F_H3	$-2.61\cdot10^{-3}$	<0.001	0.49
cpue				
Log_{10} (aan+1)	R_A8	$-3.62\cdot10^{-2}$	<0.05	0.31
Log_{10} (pfl+1)	F_H3	$-6.30\cdot10^{-4}$	<0.001	0.56
	F_H5	$-2.71\cdot10^{-2}$	<0.05	0.49
Log_{10} (rru+1)	F_H3	$-6.93\cdot10^{-4}$	<0.01	0.22
Log_{10} (ser+1)	M_A3	$4.98\cdot10^{-3}$	<0.05	0.44
Log_{10} (slu+1)	M_H17	2.24	<0.01	0.30
	F_H3	$-1.22\cdot10^{-3}$	<0.001	0.58
Shannon Diversity	M_H17	$9.44\cdot10^{-1}$	<0.05	0.51
	D_H12	$-5.88\cdot10^{-4}$	<0.01	0.54
	F_H3	$-5.31\cdot10^{-4}$	<0.001	0.62
bpue				
cca	M_H20	155.36	<0.05	0.46
	D_H12	121.15	<0.05	0.46
	F_H3	98.64	<0.05	0.48
Log_{10} (gho+1)	M_H17	2.40	<0.05	0.27
	M_H20	$-2.11\cdot10^{-3}$	<0.001	0.36
	D_H12	$-1.77\cdot10^{-3}$	<0.001	0.39
	F_H3	$-7.39\cdot10^{-4}$	<0.05	0.25
Log_{10} (pfl+1)	F_H3	$-1.20\cdot10^{-3}$	<0.001	0.54
	F_H5	$-6.86\cdot10^{-2}$	<0.01	0.50
Log_{10} (ppa+1)	F_H3	$-5.02\cdot10^{-4}$	<0.05	0.38
Log_{10} (slu+1)	M_H20	$-1.90\cdot10^{-3}$	<0.05	0.24
	D_H12	$-1.80\cdot10^{-3}$	<0.01	0.29
	F_H3	$-1.70\cdot10^{-3}$	<0.001	0.39
Shannon Diversity	F_H5	$-1.37\cdot10^{-2}$	<0.05	0.32

Table 3. Mixed Effects Models with river reach as random factor between species abundances and community metrics and hydrological indices for annual cycle. R^2 were estimated by a linear regression with fitted and real values. Only significant models are shown. *Tabla 3. Modelos de Efectos Mixtos con tramo de río como factor aleatorio entre abundancias de especies y métricas de comunidad e índices hidrológicos para los muestreos del ciclo anual. Las* R^2 *se estimaron a partir de la regresión lineal entre valores predichos y valores reales. Sólo se muestran los modelos significativos.*

Response variable	Predictor	Estimate	p	R^2
Richness	$M_{L}13$	$9.37\cdot10^{-3}$	<0.05	0.35
	$M_{H}2$	$-1.66\cdot10^{-3}$	<0.001	0.43
	$F_{L}1$	$9.56\cdot10^{-2}$	<0.05	0.34
cpue				
Log$_{10}$(aal+1)	$M_{A}12$	$4.68\cdot10^{-3}$	<0.05	0.41
Log$_{10}$(cau+1)	$M_{H}2$	$-4.51\cdot10^{-4}$	<0.05	0.31
Log$_{10}$(gho+1)	$M_{L}13$	$-8.51\cdot10^{-3}$	<0.001	0.35
	$M_{L}14$	2.51	<0.01	0.36
	$M_{H}17$	3.70	<0.001	0.40
	$F_{L}1$	$9.11\cdot10^{-2}$	<0.001	0.34
	$F_{H}3$	$-9.04\cdot10^{-4}$	<0.05	0.30
Log$_{10}$(glo+1)	$M_{L}14$	-1.84	<0.01	0.35
	$M_{A}12$	$5.25\cdot10^{-3}$	<0.01	0.33
	$M_{A}13$	$3.68\cdot10^{-3}$	<0.01	0.35
Log$_{10}$(lgi+1)	$M_{A}3$	$3.36\cdot10^{-3}$	<0.05	0.31
	$D_{L}1$	$-9.58\cdot10^{-3}$	<0.01	0.40
Log$_{10}$(lgr+1)	$M_{H}2$	$-8.09\cdot10^{-4}$	<0.001	0.41
Log$_{10}$(ppa+1)	$M_{H}20$	$-1.15\cdot10^{-3}$	<0.001	0.45
	$F_{H}3$	$-1.05\cdot10^{-3}$	<0.001	0.34
Log$_{10}$(rru +1)	$M_{H}20$	$1.04\cdot10^{-3}$	<0.01	0.21
Shannon Diversity	$M_{H}2$	$-2.63\cdot10^{-4}$	<0.05	0.30
% Alien	$M_{H}1$	$-1.59\cdot10^{-2}$	<0.05	0.47
bpue				
Log$_{10}$ (aan+1)	$M_{A}12$	$-4.59\cdot10^{-3}$	<0.01	0.33
Log$_{10}$ (cau+1)	$M_{H}2$	$-1.18\cdot10^{-3}$	<0.05	0.32
Log$_{10}$ (gho+1)	$M_{L}13$	$-1.13\cdot10^{-2}$	<0.001	0.33
	$M_{L}14$	2.40	<0.05	0.27
	$M_{H}17$	3.25	<0.01	0.27
	$F_{H}3$	$-1.12\cdot10^{-3}$	<0.05	0.23
Log$_{10}$ (glo+1)	$M_{A}12$	$6.78\cdot10^{-3}$	<0.01	0.32
	$M_{A}13$	$4.69\cdot10^{-3}$	<0.01	0.33
Log$_{10}$ (ppa+1)	$M_{H}20$	$-1.22\cdot10^{-3}$	<0.001	0.39
Log$_{10}$ (rru+1)	$M_{H}20$	$1.65\cdot10^{-3}$	<0.01	0.20
Shannon Diversity	$M_{H}2$	$-1.52\cdot10^{-4}$	<0.05	0.11
Log$_{10}$ (total+1)	$F_{H}5$	$-2.43\cdot10^{-2}$	<0.01	0.15

Table 4. Mixed Effects Models with river reach as random factor between species abundances and community metrics and hydrological indices for inter-annual samplings. R^2 were estimated by a linear regression with fitted and real values. Only significant models are shown. *Tabla 4. Modelos de Efectos Mixtos con tramo de río como factor aleatorio entre abundancias de especies y métricas de comunidad e índices hidrológicos para los muestreos inter-anuales. Las* R^2 *se estimaron a partir de la regresión lineal entre valores predichos y valores reales. Sólo se muestran los modelos significativos.*

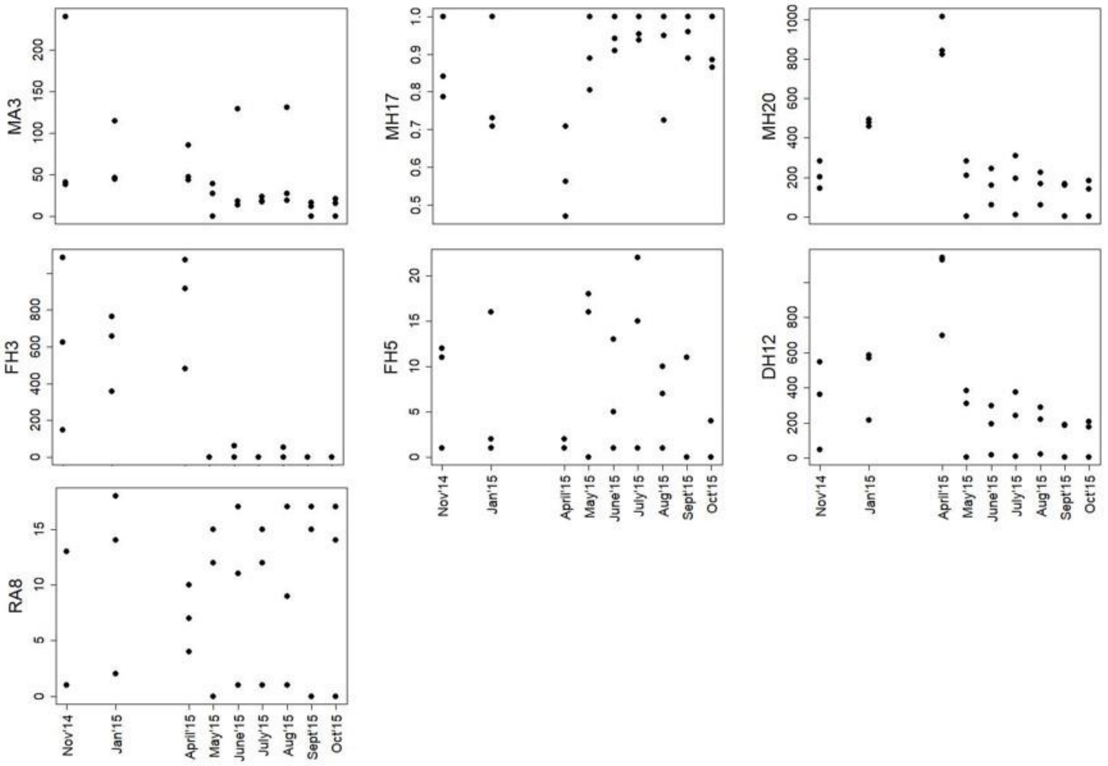

Figure 6. Hydrological indices significantly related with species abundances for the annual cycle. *Figura 6. Índices hidrológicos relacionados significativamente con abundancias de especies para el ciclo anual.*

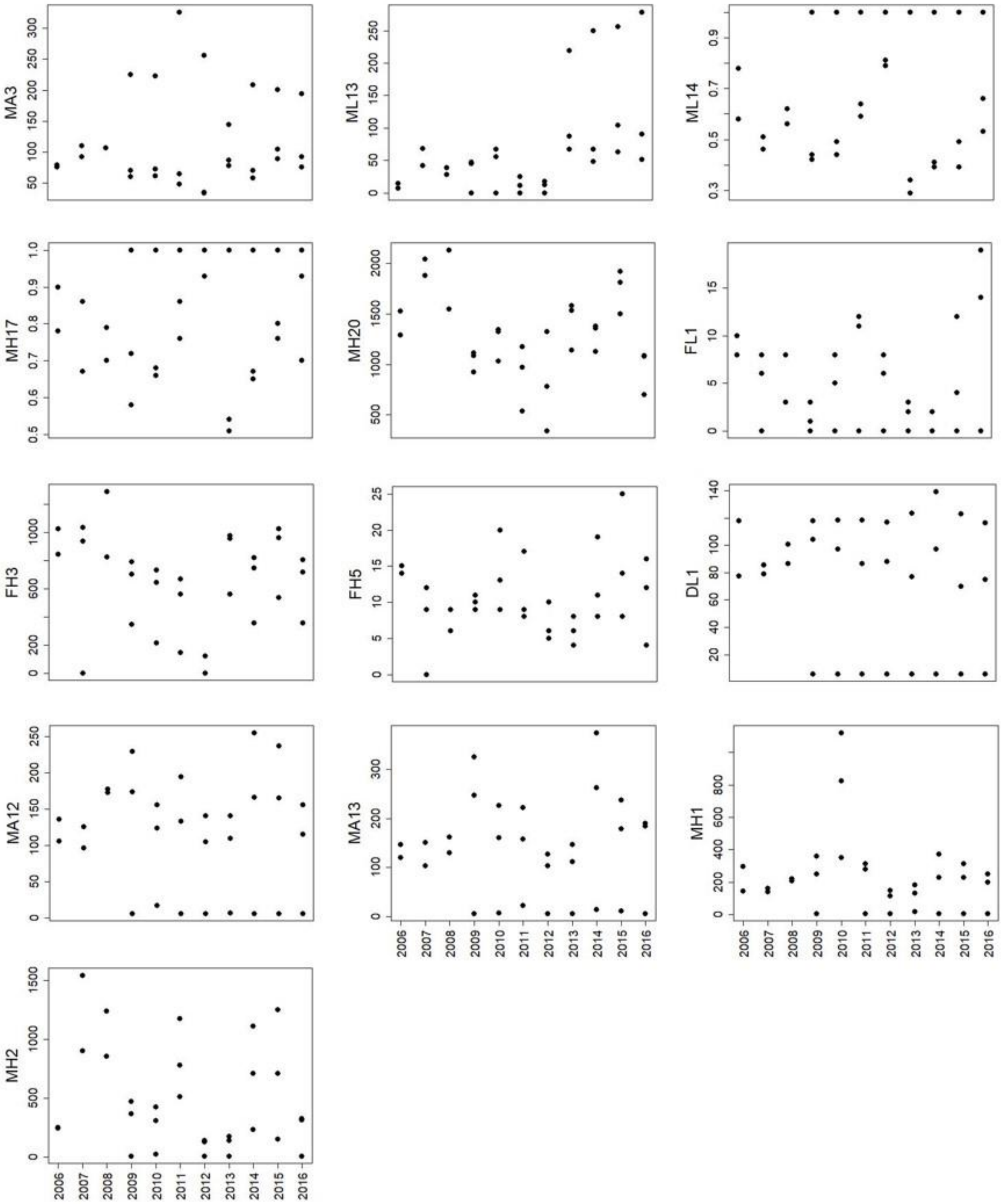

Figure 7. Hydrological indices significantly related with species abundances for the inter-annual data set. *Figura 7. Índices hidrológicos relacionados significativamente con abundancias de especies para los datos inter-anuales.*

Hydrological indices before and after dam construction

As shown in Table 5, from the 20 hydrological indices analysed, 11 presented significant differences between the natural flow regime (before dam construction) and the altered flow regime (after dam construction). Those that increased with dam regulation were (by order of magnitude of the difference): R_A8, D_L13, M_L14, M_A5 and M_H17; and those that decreased (by order of magnitude of the difference): FH3, MA12, MH20, MA13, MH2 and MH1.

index	diff	lower diff	uper diff	p-adj
M_L14	0.32	0.16	0.47	<0.001
M_H17	0.18	0.05	0.31	<0.01
M_H20	-1224.67	-1638.73	-810.6	<0.001
F_H3	-1006.48	-1338.05	-674.91	<0.001
D_L13	0.25	0.13	0.36	<0.001
R_A8	70.4	44.41	96.39	<0.001
M_A5	0.33	0.11	0.56	<0.01
M_A12	-130.32	-180.91	-79.72	<0.001
M_A13	-380.63	-533.46	-227.8	<0.001
M_H1	-423.93	-758.86	-88.99	<0.05
M_H2	-938.07	-1501.33	-374.81	<0.01

Table 5. Tukey test for comparison of hydrological indices before dam construction (1923-24/1933-34) and after dam construction (2005-06/2015-16). Only significant results of the comparison “after - before” are shown. *Tabla 5. Test de Tukey de comparación de los índices hidrológicos antes de la construcción de las grandes presas (1923-24/1933-34) y después (2005-06/2015-16). Sólo se muestran los resultados significativos de la comparación “después – antes”.*

Discussion

Community composition

As previously reported by other authors, our results showed a fish community markedly dominated by alien species (Almeida et al., 2017; Aparicio et al., 2016; Caiola et al., 2014; Elvira and Almodóvar, 2001; López et al., 2012). Taking into account all the sampling campaigns, the 50-60% in richness (margin of uncertainty that depends on the different species of mullets), the 84% in number of individuals and the 84% in biomass were alien species. During the inter-annual samplings, we observed the appearance of the European perch, a species introduced on the Ebro Basin during the decade of 1970 but that was not yet observed in this transect of the River. In our samplings the first occurrence took place in 2014 and since then its presence has been increasing, suggesting that it is in full expansion.

With respect to the freshwater blenny (*Salaria fluviatilis*) and the European eel, considered endangered fishes in Spain (Doadrio et al., 2011), they were found in the samplings but we do not have enough data to judge whether their populations presented a positive or negative evolution. In the other hand, some other native species were not found despite its potential distribution and its historical presence in the area such as the Ebro nase (*Parachondrostoma miegii),* presented by Doadrio (2002) as a broadly distributed species throughout the study area. Also the twaite shad (*Alosa fallax)* was not found, contrasting with other studies that assure that its presence has been increasing in recent years (Aparicio et al., 2016). As it was expected, none of the species considered extinct were found, i.e. the sturgeon (*Acipenser sturio)*, fished for the last time in 1970 (Almaça et al., 2000); the Allis Shad (*Alosa alosa*), not observed from 1960; and the sea lamprey (*Petromyzon marinus)*, of which there have been no observations in recent years (Aparicio et al., 2016).

Regarding the spatial distribution, the marine species were found almost exclusively in the station E01, below the Xerta weir, indicating that it constitutes an unbearable obstacle for the majority of migratory species in this area and that the fish ladder on there does not fully fulfill its fluvial connectivity function. Moreover, European perch and Largemouth black bass presented certain preference for station E06. This river reach has a special flow regime that consists in a very low and constant water flow during most of the time (about 6 $m^3 \cdot s^{-1}$) resulting in a lentic environment with a water velocity of nearly 0 $m \cdot s^{-1}$. These quiet waters constitute a preferred habitat for the mentioned species (Aparicio et al., 2016; Garvey et al., 2002; Sagnes and Statzner, 2009). In addition, station E06 is located immediately below the Flix dam, and is well established the role of reservoirs as hot spots of alien fish species dispersal in the Iberian Peninsula (Liew et al., 2016; Oliveira et al., 2018).

Influence of changing hydrology in species abundances

Annual cycle

In the first part of the analysis we explored how the immediate flow conditions affected species abundances, trying to establish seasonal flow-fish community structure interactions. High significant models were found with Eastern mosquitofish that was negatively affected by floods. These relationships revealed a diminution of abundance of Eastern mosquitofish in spring, during and after flood events, as previously observed by other authors (Alexandre et al., 2013). This was not surprising since Eastern mosquitofish is a limnophilic species that do not withstand strong water flow conditions (Murphy et al., 2015) and probably suffers displacement downstream because of strong currents. The pike-perch also resulted negatively affected by high flow conditions, in accordance with its preference for calm waters described in previous studies (Pavlicevic et al., 2016; Vehanen and Lahti, 2003). In the same way, European perch abundance was highly inversely related with F_H3, a descriptor of high flow conditions, and less significant and inversely related with F_H5 (frequency of high flood events). This means lower presence of European perch during late winter and spring, when the water velocities are higher in accordance to the greater liking for still waters reported by some authors (Lamouroux and Cattaneo, 2006; Sagnes and Statzner, 2009)

Many authors described the preference for still waters of the topmouth gudgeon (Liu et al., 2017) and the roach (Copp, 1990; Lamouroux and Cattaneo, 2006). Thus, it was not surprising that our results showed a significant negative relationship between the frequency and magnitude of high flow events (F_H3) and the abundances of these alien species. Moreover, feeding on invertebrates may result easer during low flow periods, due to the smaller preying area and the scarcity of shelters for invertebrates compared to higher water volume conditions (Alexandre et al., 2013).

On the other hand, the abundance of common carp increased with flood events, as it was found to be directly related with indices M_H20, D_H12 and F_H3, despite its limnophilic character. This result is in agreement with the findings of Macdonald and Crook (2014) that demonstrated that inundated floodplain habitats are especially important for carp reproduction, and suggested that even small flow pikes that inundate vegetated riverbank areas during spring or early summer may promote spawning and enhance survival of recruits. Brown et al (2005) observed that reproductive individuals of common carp form aggregates during winter-spring close to access points to floodplain inundation areas in order to occupy ideal habitats for spawning in the mid-Murray River in Australia. Also in other studies have been observed similar lateral movements of common carp (Jones and Stuart, 2009; Lubinsky et al., 2014).

But our results brought out other less clear relationships such as an apparent negative effect of high changing water conditions (hydrological index R_A8) in European eel abundance. Due to the complex migratory behaviour of European eel, they have a great sensitivity to the direction and magnitude of currents (Deelder, 1984; Piper et al., 2012) but far from being a limitation, they are well adapted to all types of flow conditions (Bornarel et al., 2018; Dekker, 2003). Also the rudd appeared to be positively related with M_A3, an indicator of flow variability, despite its preference for still waters (Kennedy and Fitzmaurice, 1974; Lasne et al., 2007).

Species richness and the Shannon diversity index were found to be inversely related with flood events (positive relation with M_H17 and negative relation with M_H20, D_H12, F_H3 and F_H5; Table 3). This is a direct consequence of the adverse effects that we have seen the floods have on several species of the lower Ebro: the Eastern mosquitofish, the pike-perch, the European perch, the roach and the topmouth gudgeon. As all of them are introduced, these results are further evidence that natural floods reduce the presence of introduced species in Mediterranean Rivers, and management measures should be studied to use controlled floods as tools for the conservation of autochthonous fauna, as has been proposed by other authors (Alexandre et al., 2016; Lasne et al., 2007; Ribeiro et al., 2013).

Inter-annual

In this set of data, Eastern mosquitofish presented again high significant relationships with flow, both in number of individuals and in biomass. Its population decreased in years of high flood events (positively related with M_H17 and negatively related with F_H3), and increased in years of prolonged low flow periods (positively related with M_L14 and F_L1 and negatively related with M_L13). Similarly, the topmouth gudgeon presented smaller populations in years of high flood events (highly significant negatively relation with F_H3 and M_H20, both in individuals and biomass). These results are in accordance with previous studies that associated primary production along the dry-season with reproductive periods of non-native fishes in Mediterranean rivers (Ribeiro et al., 2013) and those who observed harmful effects of floods on introduced species in this region (Bernardo et al., 2003; Caiola et al., 2014; Ribeiro et al., 2013). The roach, on the contrary, turned out to be benefited in years of high flows (significant positive relationship with yearly maximum flow) despite its limnophilic character (Copp, 1990; Lamouroux and Cattaneo, 2006). Interestingly the roach decreased during periods of high flow in the annual cycle, but when comparing years of high and low flow, the effect seems to be the opposite.

The hydrological indices that describe the magnitude of October and November flows (M_A12, M_A13 and M_H2) were found to be significantly related with several cyprinid species: the bleak and the Iberian gudgeon were benefited by high flows during these months while the goldfish and the Ebro barbel were harmed by them. The reproduction period of the cyprinids in the study area goes from April to July (Aparicio et al., 2016) and thus, high autumn flows have their effect on the individuals of early stages. A positive effect of flood events for the initial development states has been observed in other rivers, as floods provide new aquatic vegetated environments rich in shelter and food for young individuals (Agostinho et al., 2004). There are different reasons that could explain the negative effects. In one hand, short flood periods displace smallest fishes into the main river channel, where they are more prone to predation (Agostinho et al., 2004) and also the increased energy expenditure can lead to higher mortality rates (Harvey, 1987). In the other hand, there are evidences that some cyprinids in the Mediterranean rivers synchronize their biological and reproductive cycles to the hydrological regime to assure the viability of the progeny (Alexandre et al., 2015b; Magalhães et al., 2003). It seems that the fishes respond to hydrological signals (water rise together with high temperatures) that stimulate the beginning of the gonads maturation, essential phase before spawning (Bailly et al., 2008). But Alexandre et al. (2015a) observed that Iberian barbel's gonadal maturation could be delayed or even suppressed in absence of inducing floods or too short flood pulses, causing a desynchronization between the life cycle of the animal and the hydrological cycle. This imbalance between the two cycles (biological and hydrological) could be the reason why high flow rates in autumn had negative effects on some youth cyprinids survival that maybe would not have developed enough to withstand the high currents of the rainy season.

Lastly, the pumpkinseed was positive and significantly related with the coefficient of variation in daily flows (M_A3) and negatively affected by minimum annual flows (D_L1). The pumpkinseed has been described as a still-water fish for some authors (Sagnes and Statzner, 2009; Top et al., 2016) but other studies state that although its reproduction may require lentic habitat, the adults appear to be indifferent to water velocities (Klaar et al., 2004). In any case, we didn't find any potential explanation for this result and it would require further research.

Hydrological alteration indices

Hydrological indices that decreased after dam construction were descriptors of high flow events while those that increased were descriptors of low flow periods and rate of change between increasing and decreasing flows. These results reflect the typical water management for agricultural uses and urban supply that implies storing of winter floods and increased baseflows in summer for irrigation (Batalla et al., 2004). As we have seen above, some of these indices were related with species abundances and thus, we propose to use them as management tools for reducing alien species in the lower Ebro River while, at the same time, recover part of the hydrological dynamics that was lost with dam regulation. Although some of them had beneficial effects for both native and alien species and would require a deeper study, others presented clear tendencies that make them suitable to be applied as conservation measures. For example, increasing F_H3 would probably reduce topmouth gudgeon, European perch, roach, pike-perch and Eastern mosquitofish populations; a decrease in M_H17 would reduce Eastern mosquitofish and pike-perch, and an increase of M_A13 would benefit the Iberian gudgeon.

Conclusions

The conclusions of this study are: I) The lower Ebro River is dominated by alien fish, above all, introduced cyprinids. II) Several species abundances were significantly related with hydrological indices. III) Some of these hydrological indices presented differences before and after dam regulation. IV) We propose the use of these hydrological indices as management tools for the control of alien fish stocks.

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Chapter 2. Linking fish-based biological indicators with hydrological dynamics in a Mediterranean river: relevance for environmental flow regimes

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Abstract

The relationship between flow dynamics and biological communities becomes especially relevant in Mediterranean rivers. Given their natural variability and growing anthropogenic pressures, their low sections are subjected to multiple impacts. The definition of ecohydrological relationships in Mediterranean rivers may constitute a useful management tool. Historically, fishes were the first group used to assess community-level ecological quality, and different indices and metrics have been proposed. However, up to date many of these indicators have showed to be insensitive to flow regime changes or hydrological alteration. There is therefore a need to deepen into the ecohydrological relationships between such indicators and flow regimes in Mediterranean (and other) rivers. This study presents an analysis of the relationship between interannual flow regimes in the lower section of the Ebro River, defined using a set of daily and hourly hydrological indices, and ecological quality based on fish community, assessed through indices designed to fulfill the Water Framework Directive (WFD) in Europe: the Indices of Biotic Integrity in Catalan rivers (IBICAT2010 and IBICAT2b) and the new European Fish Index (EFI+). In order to identify spatiotemporal patterns, hydrological indices were computed using time periods of different amplitude and ecological quality was obtained in different transects along the river section, even within the same water units or 'water masses' (subdivisions of surface waters to fulfill the WFD in Spain). Our results showed that IBICAT2010 was the most correlated with hydrological indices, followed by IBICAT2b and EFI+. The latter showed an almost null correlation with hydrological indices, which may be caused not only by the location of the transects in which the index was originally developed (at European scale) but also because it does not consider stream typologies. Correlations among some hydrological and biological indices were observed, with temporal and spatial patterns. On one hand, daily hydrological indices showed relationship with ecological quality when they were computed using between 9 and 36 months of flow records (previous to the sampling date) whereas subdaily indices responded better to periods between 3 and 9 months of records. On the other hand, some sampling transects showed clearer relationships than others, even within the same water mass, which suggests an influence of the hydromorphologic variability on the obtained ecological quality scores.

Keywords

Ecological quality; fish indices; Ebro River; Water Framework Directive; environmental flows

Introduction

The 'natural flow regime paradigm' (Poff, 1997) defined flow dynamics as one of the main drivers of ecological properties of rivers and streams. Therefore, hydrological alteration is a potential risk for aquatic ecosystems, as it has effects on aquatic communities (Poff & Zimmerman, 2010) that may alter their characteristics even at evolutionary time scales (e.g. Mims & Olden, 2012). Specially in Mediterranean streams and rivers, subjected to a high natural hydrological variability (Gasith & Resh, 1999; Caiola et al. 2001a,b) and to many pressures frequently associated with agricultural activities, such as flow regulation by dams and water extraction for irrigation (Ferreira et al., 2007b).

The Water Framework Directive (WFD; 2000/60/EC) established the objective to achieve a 'good ecological status' in the water bodies of the European Union (including those artificial and heavily modified). With the aim of achieving this objective, the Directive requires the sub-division of surface waters into 'discrete and significant elements' or 'water bodies' (in Spain, 'water masses'). However, the Directive does not provide explicit guidance on how to identify the elements that should be regarded as 'discrete and significant' and, as a consequence, the different water bodies may present relatively heterogeneous characteristics such as the length of the stream section.

Whereas classical approaches have focused on target species to define ecohydrological relationships (e.g. Instream Flow Incremental Methodology, IFIM; Bovee & Milhous, 1978), the WFD focuses on the assessment of community-based ecological integrity. Some studies have focused on macroinvertebrates (Buffagni et al., 2005; Bennett et al., 2011; Birk and Hering, 2006, 2009), macrophytes (Birk et al., 2006; Birk and Willby, 2010) or diatoms (Birk and Hering, 2009), but the first method to assess the biotic integrity of rivers was developed specifically for fishes (e.g., Karr, 1981; Fausch et al., 1984). Fishes not only possess a higher direct socio-economic impact than other aquatic organisms but also are key indicators of ecological condition in rivers. In comparison with other taxa, they tend to be more responsive to hydromorphological disturbances (Birk et al., 2012; Marzin et al., 2012), connectivity loss (Schiemer, 2000; Sindilariu et al., 2006) and other stressors that act at wide spatial and temporal scales (Harris, 1995; Simon, 1999).

The first attempt to develop fish-based methods for ecological assessment in streams and rivers across the whole European Mediterranean basin, and fulfill the Water Framework Directive (WFD), was made within the EU-funded FAME (Development, Evaluation and Implementation of a Standardized Fish-based Assessment Method for the Ecological Status of

European Rivers; http://fame.boku.ac.at) and EFI+ (Improvement and Spatial extension of the European Fish Index; http://efi-plus.boku.ac.at/index.htm) projects. The main output of these two projects was the new European Fish Index (EFI+), the first standardized fish-based assessment applicable across nearly the whole range of European rivers (Pont et al., 2006, 2007). It is a predictive multimetric index that derives reference conditions of individual sites from abiotic environmental characteristics and quantifies the deviation between the predicted and the observed fish assemblages (Pont et al., 2006). The metrics that integrate the index are based on functional guilds that describe the main ecological and biological characteristics of fish assemblages (Logez et al., 2013). Although such index was reasonably accurate at the European scale, its applicability varied among different biogeographical regions and countries (Pont et al., 2007; Urbanic and Podgornik, 2008; Logez et al., 2010). In Spanish Mediterranean rivers, the Mediterranean Index of Biotic Integrity or IBIMED is used as a fish-based assessment method suitable for the evaluation of ecological quality. First developed for Catalan rivers under the designation of IBICAT (Index of Biotic Integrity for Catalan rivers; Sostoa et al., 2004), an improved version of this index was developed in 2010 (IBICAT2010; Sostoa et al., 2010) before being adapted to the rest of Mediterranean Spanish rivers under the designation of IBIMED. IBICAT2010 and IBIMED are similar in the Ebro River, except for the different species, ecological guilds and thresholds of the Ecological Quality Ratio (EQR) classes. They follow a type-specific method based on eight environmental variables that were selected as the best descriptors of a river classification based on historical fish distribution. More details on the EFI+ and IBIMED may be found in Segurado et al., (2014). Finally, a type-specific variant of IBICAT (IBICAT2b) uses between 4 and 8 metrics depending on river type and has been validated with environmental pressures both throughout Catalonia and the whole Ebro River Basin (Sostoa et al., 2010). IBICAT, its variant (IBICAT2b) and EFI+ have been described as correlated in the Ebro Basin (García-Berthou & Bae, 2014).

Despite aquatic communities are in general strongly affected by hydrology, most of the methods developed for the assessment of biological quality elements are largely insensitive to flow regime changes or hydrological alteration (e.g. Poff & Zimmerman, 2010; Demars et al., 2012; Friberg 2014). For example, only 40% of the methods developed for fishes are sensitive to flow modifications (Rinaldi et al., 2013). There is a need for development of biological methods to provide metrics sensitive to hydrological pressures and alteration of flow components (European Commission, 2015), which means that further investigation of the relationships between current biological indices (and metrics) and hydrological regimes results essential. Defining ecohydrological relationships in Mediterranean (and other) rivers constitutes a powerful tool for water management, in consonance with frameworks such as the Ecological Limits of Hydrological alteration (ELOHA; Poff et al., 2010).

Spatial and temporal scaling phenomena should be considered when establishing a monitoring program. The dimensions of variation change along spatial/temporal gradients of salinity, habitat complexity and productivity and among different levels of biological organization. Without an adequate evaluation of such variation, representative samples cannot be taken (Livingstone, 1987). In this context, for example, studies on juvenile salmonids and other fishes suggest that more than 5 years are needed to detect significant changes in fish abundance after physical habitat shifts (e.g. caused by hydrological variations) unless the magnitude of change in fish abundance is large (>threefold) or the treatments and controls are extensively replicated (Bisson et al., 1997; Roni et al., 2003). Attention must be paid also to temporal resolution, as the use of hourly records together with daily flows may allow distinguishing effects caused by particular flow regime characteristics such as hydro-peaking (e.g. Macnaughton et al., 2017).

The aim of this study was to test the ability of different hydrological indices to explain changes in ecological quality assessed through fishes in the lower section of a Mediterranean river (Ebro from Flix to Tortosa). IBICAT2010, IBICAT2b and EFI+ were employed in order to compare their relationship with hydrology. The relationships between IBICAT2010 and its metrics were also assessed. Different time scales (from months to years) and data resolutions (daily and hourly) were employed for the computation of the hydrological indices. We hypothesized a similar relationship among the three ecological indices and the hydrological indices used (based on García-Berthou & Bae, 2014). In addition, we expected daily hydrological indices computed with the shortest time scales (counted since the moment in which the sample was taken) not to show a relationship with ecological quality given that, as stated above, previous authors highlighted the necessity of relatively long series to detect changes in fishes after habitat shifts (Bisson et al., 1997; Roni et al., 2003). Subdaily (hourly) indices were expected to respond within shorter periods than daily indices.

Material and methods

Study area

The study was conducted in the low Ebro River, located in the NE of the Iberian Peninsula (Catalonia, Spain; Fig. 1). The study area extends from the reservoir furthest downstream (Flix) to the upper limit of the estuary (Tortosa), where the river is about 80 km long and 150 m wide. The Ebro River is 928 km long and has a drainage area of 85 550 km^2. It is the Spanish River with the highest mean annual flow and one of the most important tributaries to the Mediterranean Sea. The main land use in the basin is agricultural with more than 10 000 km^2 of irrigation, which corresponds to approximately 90% of the water usage in the basin. The whole basin is strongly regulated by nearly 200 dams, most of them built between 1940 and 1970 (Ibáñez et al., 2012a; Nebra et al., 2011). The lower Ebro hydrology, geomorphology and ecology are strongly impacted by the existence, features and operation of such dams (Ibáñez et al., 2012a, b). Apart from the Flix reservoir, upstream of the studied section, two weirs are located in the lower Ebro: Ascó and Xerta. The former is aimed to provide water for refrigeration for a nuclear plant whereas the latter is intended for irrigation. The Flix reservoir derives most of the water income through a channel that avoids the meander located directly below the dam. Only reduced water volumes are liberated intermittently to the meander, when flow overcomes the maximum allocated to produce electricity.

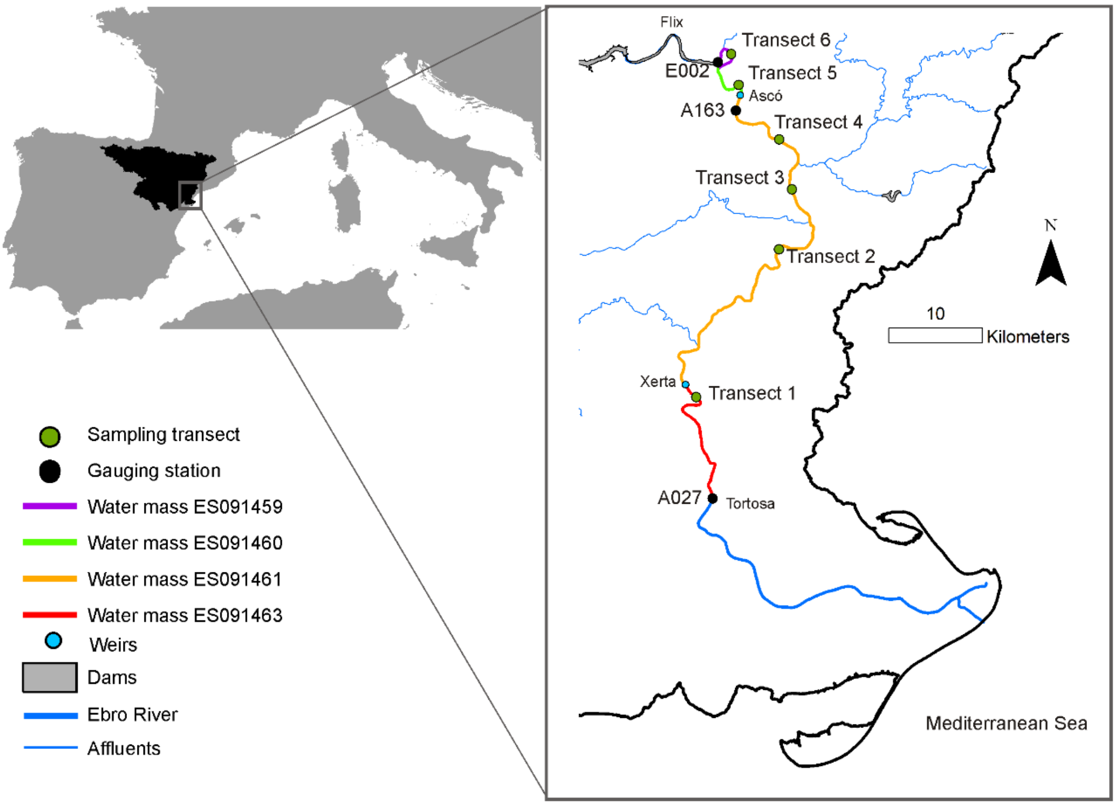

Fig. 1 Study area showing the sampling transects and gauging stations located on the water units (masses) of the low Ebro River (dams and weirs are also showed)

This river section is composed by four water masses (Fig. 1) according to the current Ebro's Water Plan: ES091463 (from the Xerta weir to Tortosa), ES091461 (from Ascó to Xerta Weir), ES091460 (from Flix to Ascó) and ES091459 (Flix meander). The water mass ES091461 is by far longer than the others (Fig. 1).

Fish and habitat data

Electrofishing data taken in six sampling transects between 2006 and 2015 allowed characterizing fish communities in the low Ebro River (Fig. 1). The sampling transects were selected randomly to avoid biases, ensuring that they covered all the hydromorphological variability in the river section observed using aerial photographs (for more details, see Caiola et al., 2014). They also provided a weighed representation of the water units or masses in the study area (as the greatest number of transects was located in the longest water mass): ES091463 (transect 1), ES091461 (transects 2, 3 and 4), ES091460 (transect 5) and ES091459 (transect 6). Transects from 1 to 5 were located in the main channel whereas transect 6 was located in the meander, directly downstream the Flix reservoir. One sample per year was taken between summer and early autumn. As the river width varies between 150 and 200 m, and CEN standards for fishing with electricity (CEN, 2003) advice that the sampling transect length should be around ten times the river width, each sampling transect had 2 km. They were sampled in 10 equidistant points located in the littoral zone (left or right bank, selected randomly). The catches of the 10 sampling points within each 2 km transect were aggregated, as the cumulative number of species in the 10 points have showed to constitute an adequate sampling effort (Caiola et al., 2014). In each transect, fish were caught with a boat-based electrofishing gear that generated up to 400 V and 10 A pulsed DC working from downstream to upstream direction. A constant distance to the river bank (5 m) and fishing time (5 min) was always maintained. Fishes from each point were kept in plastic tanks with river water for its immediate processing before the next point. The specimens were then sorted, identified to species level and counted (keeping record, if necessary, on the presence of deformities or other anomalies). Native species were returned to the river, whilst introduced alien species were eliminated with an excess of anesthetic (MS-222). The mortality of native fishes during the sampled period was negligible, restricted to only a few small individuals.

In each sampling point, habitat descriptors (depth, water velocity and riverbed dominant substrate) were recorded. Three readings of each variable were carried out. Depth was recorded with a measuring rod; water velocity was measured at a 0.6-depth with a Valeport m.001 current-meter; riverbed dominant substrate was determined visually according to a modified Wentworth scale using categories 4-10 (sand to boulder) out of the 13 particle size categories of this scale. The habitat descriptors recorded in each sampling point were expressed at transect level using the most frequent category (dominant substrate) or averaging their values (depth and water velocity). Although the former involves the loss of information, such step was necessary because one of the indices computed (EFI+) requires the dominant substrate (see details on metrics and indices below).

Fish metrics and indices

All diadromous species were removed, as the Xerta weir prevents their movement upstream and these species can only be found in the lowermost sampling transect (Fig. 1). By doing so, we ensured that this transect was comparable with the rest. Then, for each sampling transect, the IBICAT2010, IBICAT2b and EFI+ were computed. In addition, all the metrics of the IBICAT2010 based on freshwater species richness (species) and abundance (individuals) were calculated, not only those applicable to this river type (type 6). The IBICAT2b and EFI+ were calculated using the Excel templates that may be found online (http://www.invasiber.org/GarciaBerthou/ibicat2b-fish-index/ and http://efi-plus.boku.ac.at/software/insert_data.php, respectively). All indices and metrics obtained are shown in Table 1. They were also computed combining the transects 2, 3 and 4 in order to test ecohydrological relationships at a greater spatial scale for the water mass ES091461 (given its greater length) aggregating the captures collected in the three transects and correcting the result by the sampled area (to take into account the greater sampling effort). By doing so, we tested if such alternative approach may produce more accurate results in long water masses.

Type	Acronym	Description
Index	IBICAT2010	Index of Biotic Integrity in Catalan Rivers (2010)
	IBICAT2b	Index of Biotic Integrity in Catalan Rivers (2b)
	EFI+	European Fish Index
Metric	CPUEI	Density of alien individuals
	NIN_Pis	Density of native piscivorous individuals
	NIT_Inv	Density of invertiborous individuals
	NIT_Omn	Density of omnivorous individuals
	NIT_Rhe	Density of reophilic individuals
	NSI_Tol	Number of alien tolerant species
	NSN_Int	Number of native intolerant species
	NSN_Lit	Number of native litophilous species
	PIT_DELT	Percentage of individuals with deformities/lesions/parasites
	Pit_Int	Percentage of intolerant individuals
	PIT_Omn	Percentage of omnivorous individuals
	PII_Inv	Percentage of alien invertiborous individuals
	PSN_Lit	Percentage of native litophilous species
	PSN_Tol	Percentage of native tolerant species

Table 1. Indices and metrics (which belong to IBICAT2010) computed to assess ecological quality

Hydrological records

Flow series for the period 2000-2015 were obtained by request to the automatic network of gauging stations (SAIH, 'Sistema Automático de Información Hidrológica') in the Ebro Basin. The series consisted on 15-minutal records belonging to two stations (Fig. 1): Tortosa (A027) and Ascó (A163); and daily data belonging to one station: Flix (E002). Using these series, hourly and daily series were generated in order to compute hydrological indices to define the main characteristics of flow regimes. Tortosa (A027) was assigned to the water mass ES091463 (transect 1), Ascó (A163) was assigned to the water masses ES091461 (transects 2, 3 and 4) and ES091460 (transect 5) and Flix (E002) was assigned to the water mass ES091459 (transect 6).

Hydrological indices

A set of hydrological indices (based on Olden & Poff, 2003) were computed from daily data to characterize flow regimes (Table 2). Such indices were complemented with other indices important to examine the ecological response of fish community, based on hourly data

(Bevelhimer at al., 2014). All hydrological indices were computed using 12 months previous to sampling and, then, sequentially smaller or broader periods of hydrological records (3, 6 and 9 or 24, 36 and 48 months since the sampling date, respectively). Given their more accurate temporal resolution, those indices based on hourly data were also computed using a 1-month period of records. Finally, both daily and hourly indices were calculated using the flow records of the hydrological year (October-September) in which the samples were taken, as they are used as standard temporal frame for water management.

Relationships between hydrological regimes and ecological quality

First, Pearson correlations were used to search relationships among flow regimes, represented through the set of hydrological indices (Table 2) and variables (water velocity and depth), and fish communities, characterized using fish indices (Table 1). The hydrological indices computed with 12 months of records were used for this step, in order to encompass the previous annual cycle of the sampled fish communities. Pearson correlations between the IBICAT2010 and its metrics (Table 1) were also calculated in order to explore their relationship. Only correlations greater than 70% were retained in both cases. Second, using only the hydrological indices retained in the previous step and the fish index that showed most of these correlations (the most sensitive), General Linear Models (GLMs) were employed in order to test the significance of ecohydrological relationships. Correlations between the most sensitive fish index and the hydrological indices computed with all periods of record were also computed to validate the choice of the selected period. The adjustment of the GLMs and the relationships between independent and dependent variables were examined to validate the results and determine the presence of potential breakpoints. The assumptions of Gaussian models were verified.

All analyses were developed in R (R Core Team, 2017).

Data	Acronym	Name	Meaning
Daily	MA3	Variability in daily flows	Coefficient of variation in daily flows
	MA44	Variability across annual flows	Difference between percentiles 90 and 10 divided by median
	ML13	Variability across minimum monthly flows	Coefficient of variation in minimum monthly flows
	ML14	Mean of annual minimum flows	Mean of the lowest annual daily flow divided by median
	ML23	Low flow discharge	Mean of the percentile 25 divided by median daily flows
	MH20	Mean annual maximum flows	Mean of the annual maximum flows
	FL1	Low flood pulse count	Number of drops below the percentile 25
	FL3	Frequency of low flow spells	Total number of low spells (5% of mean daily flow)
	FH3	High flood pulse count	Average of daily flows above 3 times the median daily flow
	FH5	Flood frequency	Number of flow events greater than the median per year
	DL1	Annual minima of daily discharge	Magnitude of minimum annual daily flow
	DL13	Mean of 30-day minima of daily discharge	Mean annual 30-day minimum divided by median flow
	DH12	Means of 7-day maxima of daily discharge	Mean annual 7-day maximum divided by median flow
	TL1	Julian date of annual minimum	Julian date of annual minimum
	RA8	Reversals	Number of changes between rising and falling periods
	MA5	Skewness in daily flows	Mean daily flows divided by median daily flows
	MA12	Mean October flow	Average flow in October
	MA13	Mean November flow	Average flow in November
	MH1	Mean October high flow	Maximum monthly flow in October
	MH2	Mean November high flow	Maximum monthly flow in November

Table 2 Hydrological indices used to characterize flow regimes

Data	Acronym	Name	Meaning
Subdaily	dmin	Daily minimum	Lowest measured flow during a 24-h period
	dmax	Daily maximum	Highest measured flow during a 24-h period
	dD	Daily delta or range	Difference between daily minimum and maximum
	dSD	Daily standard deviation	Standard deviation of the 24 hourly flow values
	dramp	Maximum hourly ramp rate	Greatest hourly incremental change during 24 hours
	dpath	Daily path length	Sum of the absolute values of hour-to-hour changes in flow
	drev	Reversals	Number of changes between rising and falling periods
	drf	Rise and fall counts difference	Difference between the number of hours of rising and falling flow
	dstD	Daily standardized delta	Daily delta divided by the daily mean over each 24-h period
	dAstD	Annually standardized delta	Daily delta divided by the mean annual daily flow
	dCv	Coefficient of variation	Daily standard deviation divided by mean annual daily flow
	dstMHramp	Standardized maximum hourly ramping rate	Maximum daily ramp rate divided by the mean annual daily flow
	dflash	Richard's Barker flashiness index	Daily path length of oscillations divided by the daily mean

Table 2 (cont.)

Results

Fish indices and metrics

Eighteen fish species were caught in the study area, most of them belonging to the families Cyprinidae, Poeciliidae and Angillidae (Annex I). Both IBICAT2010 and IBICAT2b showed values that oscillated between the two worst categories (‘poor’ and ‘bad’) for all transects and years, although the range of the numeric values obtained was different (from 1.17 to 7.29 for the former and from 1 to 2 for the latter). The EFI+ does not have categories but, taking into consideration that an undisturbed transect would have an index value close to 0.80 whereas a highly disturbed transect would show a value lower than the 25% quantile of the index distribution for undisturbed transects, it may be inferred that all transect and years can be considered as disturbed. All values were lower than 0.20. Finally, the correlation between IBICAT2010 and the other two indices (IBICAT2b and EFI+) was negligible (0.11 and -0.10, respectively) whereas the correlation between them was slightly greater and negative (-0.43).

Among the three metrics that must be used in this type of river (type 6) to calculate the IBICAT2010, no native intolerant species (NSN_Int) was found. The percentage of individuals of invertivorous alien species (PII_Inv) was correlated with the IBICAT2010 in all transects (Table 3) whereas the density of individuals belonging to alien species (CPUEI) only was correlated in transect 5. Beyond these metrics, the density of invertivorous (NIT_Inv) was correlated with IBICAT2010 in all transects except transect 2, whereas the percentage of omnivorous (PIT_Omn) showed correlation in all transects except transects 1 and 6. Other metrics showed correlation only in a few transects, such as the percentage of tolerant native species (PSN_Tol), whereas the density of native piscivorous (NIN_Pis), the percentage of individuals with deformities (PIT_DEL) and the percentage of native lithophyte species (PSN_Lit) did not show any correlation with the IBICAT2010.

Acronym	Description	Transect 1	Transect 2	Transect 3	Transect 4	Transect 5	Transect 6	Transect 2, 3 & 4
CPUEI	Density of alien individuals	-0,58	0,00	0,33	0,19	**-0,83**	-0,43	0,46
NIN_Pis	Density of native piscivorous individuals	-0,17	-0,17	0,20	-0,18	0,42	0,49	-0,24
NIT_Inv	Density of invertiborous individuals	**-0,92**	-0,57	**-0,90**	**-0,79**	**-0,84**	**-0,95**	**-0,81**
NIT_Omn	Density of omnivorous individuals	-0,09	0,25	0,65	0,54	0,24	0,03	**0,77**
NIT_Rhe	Density of reophilic individuals	-0,29	0,57	0,30	**0,75**	0,55	0,39	**0,80**
NSI_Tol	Number of alien tolerant species	0,02	-0,37	-0,03	0,11	0,24	0,23	0,03
NSN_Lit	Number of native litophilous species	0,11	0,01	0,25	**0,85**	0,31	**0,74**	0,23
PIT_DELT	Percentage of individuals with deformities/lesions/parasites	0,02	0,31	0,36	-0,05	-0,19	-0,31	-0,07
PIT_Omn	Percentage of omnivorous individuals	0,34	**0,84**	**0,80**	**0,80**	**0,72**	0,26	**0,89**
PII_Inv	Percentage of alien invertiborous individuals	**-0,96**	**-0,95**	**-0,99**	**-0,98**	**-0,98**	**-0,97**	**-0,97**
PSN_Lit	Percentage of native litophilous species	0,31	0,49	0,41	0,61	0,51	0,66	0,62
PSN_Tol	Percentage of native tolerant species	0,39	-0,33	0,12	0,24	**0,75**	**0,76**	-0,34

Table 3 Pearson correlations between the IBICAT2010 and its metrics in the sampled transects. Correlations greater than 70% are marked in bold. Correlations for the two indices based on intolerant species or individuals are not showed because they were absent in samples

Ecohydrological relationships

Among the transects located in the main channel (transects from 1 to 5), the transect 4 showed the best relationships between IBICAT2010 and hydrological indices (calculated using the 12 months previous to fish sampling), as the majority of the correlations were greater than 70% (Table 4). Such indices were the variability across minimum monthly flows (ML13), the mean of annual minimum flows (ML14), the low flow discharge (ML23), the high flood pulse count (FH3) and the standardized maximum hourly ramping rate (dstMHramp). All of them were negatively related to the IBICAT2010 except ML13 and FH3. The rest of transects in the main channel also showed correlations with some of these indices. Among them, the other transects located in water mass ES091461 (transects 2 and 3) showed a lower presence of elevated correlations. Transect 2 only showed correlation with the skewness in daily flows (MA5), whereas transect 3 did not show any correlation greater than 70%. Transect 1 did not show any correlation beyond such threshold either. Not all indices could be calculated in the transect out of the main channel (transect 6, located in the Flix meander), given its particular flow regime (with many days with extremely low flows). However, ML13 and FH3 were also correlated with IBICAT2010, together with the variability in daily flows (MA3), the variability across annual flows (MA44), the mean annual maximum flows (MH20) and the number of reversals (RA8).

Acronym	Name	Transect 1	Transect 2	Transect 3	Transect 4	Transect 5	Transect 6	Transect 2, 3 & 4
z	Depth	0,59	-0,09	-0,60	**-0,86**	-0,08	**-0,84**	**-0,72**
v	Velocity	0,40	0,62	-0,49	-0,08	-0,23	-0,29	0,03
MA3	Variability in daily flows	-0,37	0,42	0,36	0,03	0,52	**-0,75**	0,23
MA44	Variability across annual flows	-0,05	0,52	0,54	0,33	0,57	**0,71**	0,51
ML13	Variability across minimum monthly flows	0,14	0,35	0,19	**0,82**	**0,76**	**0,77**	0,60
ML14	Mean of annual minimum flows	-0,22	-0,40	-0,30	**-0,93**	-0,59	N/A	**-0,71**
ML23	Low flow discharge	-0,49	-0,38	-0,24	**-0,88**	-0,61	N/A	-0,63
MH20	Mean annual maximum flows	-0,20	0,51	0,40	0,24	0,69	**0,97**	0,37
FL1	Low flood pulse count	-0,43	0,34	0,41	0,33	0,26	N/A	0,42
FL3	Frequency of low flow spells	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FH3	High flood pulse count	0,01	0,67	0,50	**0,78**	**0,78**	**0,79**	**0,81**
FH5	Flood frequency	-0,03	0,05	0,24	0,21	-0,03	-0,51	0,17
DL1	Annual minima of daily discharge	0,49	0,26	0,31	0,55	0,08	N/A	0,52
DL13	Mean of 30-day minima of daily discharge	-0,26	0,24	0,18	-0,37	0,18	0,31	0,03
DH12	Means of 7-day maxima of daily discharge	-0,18	0,36	0,44	-0,29	0,15	0,50	0,05
TL1	Julian date of annual minimum	-0,41	0,32	0,60	-0,01	0,02	-0,48	0,30
RA8	Reversals	0,26	-0,34	-0,06	-0,17	-0,21	**0,76**	-0,30
MA5	Skewness in daily flows	-0,45	**-0,73**	-0,60	-0,24	-0,42	N/A	-0,65
MA12	Mean October flow	-0,53	0,30	0,40	0,53	0,20	0,22	0,57
MA13	Mean November flow	0,04	0,16	0,25	0,59	0,17	-0,23	0,46
MH1	Mean October high flow	0,58	-0,39	-0,34	0,51	0,14	0,22	-0,10
MH2	Mean November high flow	-0,40	0,06	0,20	-0,13	0,14	-0,23	0,07

Table 4 Pearson correlations between the IBICAT2010 and hydrological variables (habitat descriptors, daily and subdaily indices, respectively) computed using 12 months of records. Correlations greater than 70% are marked in bold. Indexes not computed due to absent or constant flow records are marked as 'N/A'

Acronym	Name	Transect 1	Transect 2	Transect 3	Transect 4	Transect 5	Transect 6	Transect 2, 3 & 4
dmin	Daily minimum	0,52	0,25	0,16	0,67	0,28	N/A	0,57
dmax	Daily maximum	-0,31	0,40	0,23	-0,09	0,55	N/A	0,16
dD	Daily delta or range	0,13	0,34	0,56	0,63	0,22	N/A	0,64
dSD	Daily standard deviation	0,15	0,36	0,57	0,66	0,23	N/A	0,67
dramp	Maximum hourly ramp rate	-0,13	-0,13	-0,14	-0,64	-0,01	N/A	-0,46
dpath	Daily path length	0,11	0,28	0,52	0,55	0,13	N/A	0,58
drev	Reversals	0,16	0,27	0,49	0,54	0,21	N/A	0,57
drf	Rise and fall counts difference	0,32	-0,12	-0,08	0,15	-0,21	N/A	0,04
dstD	Daily standardized delta	-0,07	0,17	0,50	0,41	-0,04	N/A	0,45
dAstD	Annually standardized delta	0,17	-0,07	0,37	-0,06	-0,40	N/A	0,07
dcv	Coefficient of variation	0,16	-0,05	0,41	-0,01	-0,41	N/A	0,13
dstMHramp	Standardized maximum hourly ramping rate	-0,19	-0,41	-0,43	**-0,90**	-0,46	N/A	**-0,75**
dflash	Richard's Barker flashiness index	-0,15	0,10	0,45	0,27	-0,16	N/A	0,35

Table 4 (cont.)

Finally, when transect 2, 3 and 4 were combined to represent the water mass ES091461, an intermediate number of indices presented high correlations: the mean of annual minimum flows (ML14), the high flood pulse count (FH3) and the standardized maximum hourly ramping rate (dstMHramp). Water depth was correlated with IBICAT2010 in transects 4 and 6, as well as in the combination of transects 2, 3 and 4.

IBICAT2b showed patterns similar to IBICAT2010 in terms of the hydrological indices that were correlated with the index but there were fewer correlations greater than 70% (Annex II). The EFI+ practically did not show correlations with the selected hydrological variables greater than 70%, except a couple of indices (MA12 and MH1) in the transect out of the main channel (transect 6).

Given the stated results, the hydrological variables selected because of their high correlation with fish indices (depth, ML13, ML14, ML23, FH3, dstMHramp) were introduced in General Linear Models (GLMs) one at a time, as they were also correlated among them, using the IBICAT2010 as predicted variable. Statistically significant results and a degree of adjustment that varied between 50 and 75% were obtained for most hydrological variables in transects 4 and 5, as well as in the combination of transects 2, 3 and 4 (Table 5). In the main channel, the significant models presented significant intercepts only when they showed an inverse relationship between the IBICAT2010 and the considered variable. In particular, water depth, the mean of annual minim flows (ML14), the low flow discharge (ML23) and the standardized maximum hourly ramping rate (dstMHramp). These variables showed potential breakpoints in transect 4 (Fig. 2): depth ≈ 2; ML14 ≈ 0.6; ML23 ≈ 0.7 and dstMHramp ≈ between 3 and 4. Such breakpoints would allow, moreover, identifying a threshold between the two different ecological categories observed (‘poor’ and ‘bad’).

Acronym	Name	Model	Transect 1	Transect 2	Transect 3	Transect 4	Transect 5	Transect 6	Transect 2, 3 & 4
z	Depth	p	0,11	0,81	0,07	***0,00	0,82	*0,02	*0,02
		R^2	0,20	-0,12	0,28	0,71	-0,12	0,66	0,47
		p_int	0,59	*0,03	***0,00	***0,00	0,13	***0,00	***0,00
		slope	+	-	-	-	-	-	-
ML13	Variability across minimum monthly flows	p	0,92	0,32	0,59	***0,00	**0,01	*0,04	0,06
		R^2	-0,12	0,01	-0,08	0,63	0,52	0,51	0,29
		p_int	***0,00	**0,01	**0,01	0,43	0,10	**0,01	*0,03
		slope	+	+	+	+	+	+	+
ML14	Mean of annual minimum flows	p	0,34	0,25	0,40	***0,00	0,08	N/A	*0,02
		R^2	0,00	0,05	-0,03	0,86	0,26	N/A	0,45
		p_int	***0,00	**0,01	0,02*	***0,00	***0,00	N/A	***0,00
		slope	-	-	-	-	-	N/A	-
ML23	Low flow discharge	p	0,09	0,28	0,51	***0,00	0,06	N/A	*0,05
		R^2	0,23	0,04	-0,06	0,75	0,30	N/A	0,32
		p_int	***0,00	0,03*	0,07	***0,00	**0,01	N/A	***0,00
		slope	-	-	-	-	-	N/A	-
FH3	High flood pulse count	p	0,87	*0,04	0,14	**0,01	**0,01	*0,03	***0,00
		R^2	-0,12	0,37	0,15	0,56	0,57	0,55	0,62
		p_int	*0,02	0,60	0,38	0,34	0,64	0,20	0,84
		slope	+	+	+	+	+	+	+
dstMHramp	Standardized maximum hourly ramping rate	p	0,55	0,24	0,21	***0,00	0,18	N/A	**0,01
		R^2	-0,07	0,06	0,09	0,78	0,11	N/A	0,51
		p_int	***0,00	***0,00	***0,00	***0,00	***0,00	N/A	***0,00
		slope	-	-	-	-	-	N/A	-

Table 5 GLM models between the IBICAT2010 and hydrological variables (p: p value; R^2: coefficient of determination; p_int: p value of the intercept; slope: sign of the coefficient). Significant p-values are highlighted with asterisks (*: ≤0,05; **: ≤0,01; ***: ≤ 0,001). Indexes not computed due to absent or constant flow records are marked as 'N/A'

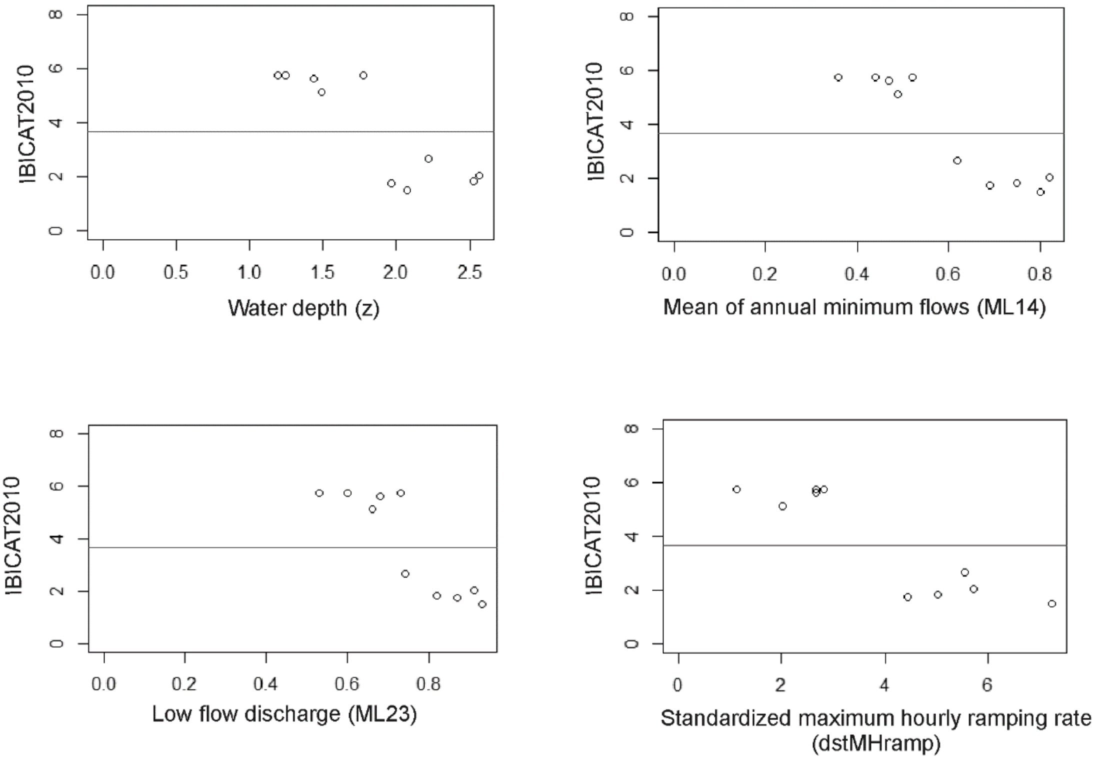

Fig. 2 Potential ecological quality breakpoints detected through the Index of Biotic Integrity in Catalan rivers 2010 (IBICAT2010), using the transect 4 and the 12-month period. The horizontal line separates the 'poor' and 'bad' status

Effects of temporal scale on ecohydrological relationships

Daily hydrological indices that showed a correlation greater than 70% with IBICAT2010 when they were calculated with the 12 months previous to the sampling date also did when they were computed using the previous 9 months, the hydrological year and, to a lesser extent, the previous 24 and 36 months (Annex III). Most of these correlations were found in transect 4. No correlation greater than 70% was found for any of these indices when they were computed using the 48 months previous to the sampling date, except when ML13 was computed combining transects 2, 3 and 4. Some subdaily indices (dpath and drev) also showed correlations greater than 70% (or close) when they were computed using flow series of 3, 6 and 9 months, but not using 1 month, the hydrological year or greater periods (24, 36 and 48 months). Within the 3-month period, the number of subdaily hydrological indices with a correlation greater than 70% with IBICAT2010 was greatest (as also included dstD, dAstD, dCV and dflash).

Apart from these general patterns, other indices showed isolated correlations using other periods of computation (for example, RA8 using the 36 months previous to the sampling date or dAstD and dCV using the previous 36 and 48 months).

Discussion

This study analyses the relationship between flow regimes and fish-based ecological quality in the low Ebro River, a Mediterranean watercourse subjected to severe anthropogenic stress. The approach used has several advantages: 1) it allowed comparing the results of different fish indices and their degree of response to specific hydrological indices; 2) the effect of taking into consideration distinct temporal scales and resolution for the hydrological indices was tested. Such information may constitute a valuable tool to provide sustainable management rules for this river in particular and for similar rivers in Mediterranean (or other) areas, within a type-specific management based on frameworks such as the Ecological Limits of Hydrologic Alteration (ELOHA; Poff et al., 2010).

Quality indices and their response to hydrological regimes

Contrary to expected, the three fish indices showed different responses to hydrological regimes. The correlation among IBICAT2010, IBICAT2b and EFI+ and the lowest performance of IBICAT2010 in the Ebro Basin highlighted by García-Berthou & Bae (2014) disappeared when only the lower Ebro was considered. The IBICAT2010 showed to be the most effective index to find relationships with flow regime. Its wider range of values may have contributed to find statistical relationships with hydrological indices more effectively. Similar values within a narrower range made more difficult the detection of statistical relationships using IBICAT2b. EFI+ showed the lowest performance, as practically no relationship with hydrological indices was found. This may be due to three possible reasons. First, whereas IBICAT2010 and IBICAT2b were developed in the lower Ebro (among other Catalan rivers), EFI+ was developed in rivers and streams of northern European latitudes. Second, EFI+ must be used with caution when transects have been sampled by boating, especially for cyprinids such as in this case (EFI+ CONSORTIUM, 2009). Third, transect selection criteria are exigent in terms of the length that must be sampled. For a large river such as the Ebro (catchment >1000 km^2), the index may require sampling transects in river segments with a length of 10 kilometers (EFI+ CONSORTIUM, 2009), which was not done in this case because sampling was developed following CEN standards (CEN, 2003). Segments five times longer would have been a logistic problem to develop the sampling procedure. In fact, EFI+ values may decrease with increasing sampling area despite the higher observed richness, as the expected values of metrics are higher (Almeida et al., 2017). This index based on a predictive model built on environmental variables, instead of in river typologies, makes more likely the presence of 'noise' (e.g. assessing the substrate dominance in sampling sites). On the contrary, a regional fish index does not depend on the sampled area, because it does not use a predictive model (Almeida et al., 2017). The low performance of the EFI+ observed in this study seems to indicate that it is not suitable for the lower Ebro. This deserves further research, as the corresponding water agency ('Confederación Hidrográfica del Ebro') has used this index to evaluate the ecological status of streams and rivers (http://www.chebro.es).

The degree of correlation between IBICAT2010 and the metrics that must be employed in the corresponding river type (type 6) depends on the considered metric. The percentage of individuals of invertivorous alien species (PII_Inv) is responsible for most of the variation in the values obtained for the IBICAT2010. Similarly, the density of invertivorous (NIT_Inv) also showed a great correlation with the index. This importance of invertivorous to assess ecological quality is coherent with studies that highlighted their sensitivity to disturbance both in Iberian (Ferreira et al., 2007a) and non-Iberian (e.g. Tejerina-Garro & Merona, 2010) rivers. Similarly, the fact that another metrics conceived to be calculated in other river types, such as the percentage of omnivorous (PIT_Omn), was also correlated with IBICAT2010 shows their potential to be used also in this river type (type 6), under the assumption that disturbance promotes opportunistic omnivorous diets (e.g. Tejerina-Garro & Merona, 2010).

The differences in the ecohydrological relationships observed in transects within the same water mass, involves different abilities to properly represent the ecological status of such water masses, and to assess the relationships between such status and environmental flow regimes. In this context, assessing the potential effect of spatial and temporal scales results essential.

Spatial and temporal scales and their effect on the assessment of ecohydrological relationships

This study supports the conclusion that temporal and spatial dimensions of a given sampling effort can have a decisive effect on the evaluation of physical, chemical and biological factors (Livingstone, 1987). The relationships among ecological and hydrological indices herein presented provide an evaluation of spatiotemporal variation in ecohydrological relationships that may result useful in low sections of Mediterranean (or other) rivers. In addition, it allows completing previous studies that did not find spatial differences among transects using macroinvertebrate and diatom communities (Quevedo et al., 2018) or assessed the effect of spatial variation on fish community whereas they stated that more effort should be put into sampling replicate sites and understand scales of temporal variation (Gray et al., 2009). Details on the spatial and temporal patterns detected in our study area are discussed below.

The fact that the number of hydrological indices correlated with IBICAT2010 varied among transects, even in those transects within the same water mass, evidences the spatial dependence of results. Transects were selected to be representative of the hydro-geomorphic variability of the lower Ebro River (Caiola et al. 2014), and the differences in the correlations obtained could be related to physical habitat. Intricate patterns of habitat complexity among other factors (recruitment features of individual species, predator-prey interactions and competition; Livingston et al. 1985) may have influenced the different results obtained in each transect. Depending on the objective of the corresponding monitoring program, assessing the ecological quality of long water masses through the combination of more than one transect may produce more representative results at water mass level. In this study, combining the transects 2, 3 and 4 produced ecohydrological relationships more representative of the water mass ES091461. Although such approach results more accurate at water mass level, understanding the responses of the integrant transects and the specific patterns of habitat complexity that mediate such relationships results essential. This is particularly important considering the potential breakpoints observed in the transect 4.

The relationships between flow regimes and ecological properties may be assessed at distinct temporal scales (monthly or annually) and resolutions (using daily or subdaily hydrological indices), which becomes essential for water management (especially in regulated rivers). Whereas previous studies established that a minimum of five years is required to observe changes in fish abundance after habitat shifts (Bisson et al., 1997; Roni et al., 2003), our results indicate that shorter temporal scales (9-36 months, including the hydrological year) are accompanied by changes in ecological quality scores (although not necessarily in ecological categories). Subdaily indices influence fish indices in shorter periods (some months of flow records), as they operate at a finer temporal resolution. The fact that indices computed with subdaily data provided significant ecohydrological relationships is relevant for managing activities such as hydropower generation, which causes flow variations within this temporal resolution. According to our results, these subdaily ecohydrological relationships will be more robust during the trimester previous to sampling, although they may be observed before.

Relevance for the establishment of ecological flow regimes in the Ebro Basin

Our study supports previous publications stating that the effect of flow regimes on biological communities is due mainly to the magnitude and variability of flows (see Belmar et al., 2013a for an example with macroinvertebrates), given that flow extremes, their relationship with mean flows and the period in which such variations take place showed to be related to fish-based ecological quality. Flow regime extremes are important for fish communities because they are responsible for the instability of habitat conditions, which plays in favor of opportunistic species. In fact, a recent study (Sabo et al., 2017) has used flow variance to design an algorithm for a managed hydrograph to explore the effect of designed flows on fishery yield. Our study shows that attention must be paid to the specific hydrological metrics used. Less than half a dozen (depending on the considered transect) out of the 19 daily indices showed a correlation greater than 70%. Similarly, two out of 13 subdaily indices provided such correlations with different time scales. Therefore, the choice of the specific indices to assess ecohydrological relationships results critical for water management. In this context, the hydrological indices used to define environmental flows by the Water Administration ('Confederación Hidrográfica del Ebro') may require revision. The study developed in the Ebro Basin (MARM, 2008) used six hydrological indices based on the magnitude and variability of flows. Although such study involved the use of habitat simulation in a posterior stage, this relatively reduced number of hydrological indicators was not tested against ecological data, as they are variations of simple metrics available in bibliography.

Future challenges

Further research may allow improving the way to predict composition and structure of aquatic communities from hydrological variables. Changes on ecosystems caused by external forces tend to occur in synchrony rather than as individual pressures (Ormerod et al., 2010). Therefore, there is a need to improve our knowledge of the links between changes in flow, channel morphology and water quality, and to assess whether impacts are additive, synergistic or antagonistic. This may be achieved in future extending field data collection to incorporate more sites where single and multiple pressures exist or undertaking manipulative experiments in which single variables are changed whilst others are held constant (Acreman et al., 2014). In addition, given that alterations to single external pressures (such as flow) may interact in complex ways with internal processes (such as biotic interaction and trophic relationships that govern flows of energy and carbon and thus also control ecosystem type, health and status), there is a need to address the challenges of combining flow effects with internal ecosystem dynamics. Finally, the consequences of extreme hydrological events must also be taken into consideration. Flood and low flow events may cause greater impacts in river ecosystems than changes in flow means (Woodward et al., 2016), as the magnitude and frequency of high and low flows regulate numerous ecological processes (Poff et al., 1997). By being able to define relationships between hydrological extremes and fish community, we could also establish relationships with other factors. For example, Belmar et al., (2018) showed that mature forests were associated with less extreme flow events, which might allow defining connections between land cover at catchment scale and fish communities.

From a management perspective, the set of hydrological indices presented in this study may be used to estimate the effectiveness of environmental flow regimes already designed or to propose management strategies. Nevertheless, the assessment and implementation of environmental flow regimes in low river sections and estuaries would require broader analyses to take into consideration additional factors (Ibáñez & Prat, 2003) such as other organisms (e.g. birds), impacts on socioeconomic activities (e.g. coastal fisheries) and even other types of flows (solid flows or sediments). In this context, hydrological series estimated under different scenarios based on forecasted climatic tendencies and management strategies would allow anticipating future values of hydrological indices and, therefore, changes in ecological quality and socioeconomic activities.

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Annexes

Annex I CPUEs or captures per effort unit (individuals/hectare) of the fish species sampled, sorted by family (in bold letter)

	CPUEs
Anguillidae	**2064**
Anguilla anguilla	2064
Atherinidae	**85**
Atherina boyeri	85
Blenniidae	**391**
Salaria fluviatilis	391
Centrarchidae	**570**
Lepomis gibbosus	545
Micropterus salmoides	25
Cyprinidae	**16028**
Alburnus alburnus	12056
Carassius auratus	70
Cyprinus carpio	373
Gobio lozanoi	152
Luciobarbus graellsii	85
Pseudorasbora parva	982
Rutilus rutilus	1517
Sander lucioperca	378
Scardinius erythrophthalmus	71
Squalius laietanus	344
Poeciliidae	**6261**
Gambusia holbrooki	6261
Siluridae	**184**
Silurus glanis	184

Annex II Pearson correlations between the hydrological variables computed using 12 months of records and a) IBICAT2b or b) EFI+ (correlations greater than 70% are in bold letter). Meaning of indices in Table 2. Indexes not computed due to absent or constant flow records are marked as 'N/A'

a)	IBICAT2b						
Hydrological variable	Transect 1	Transect 2	Transect 3	Transect 4	Transect 5	Transect 6	Transect 2, 3 & 4
Depth	0,03	-0,44	-0,01	**-0,80**	-0,43	-0,40	-0,44
Velocity	-0,11	-0,48	0,07	0,09	-0,45	0,01	0,03
MA3	0,00	-0,63	-0,57	-0,15	-0,17	**-0,93**	-0,65
MA44	0,44	-0,12	-0,33	0,13	0,23	**0,85**	-0,18
ML13	**0,71**	0,34	-0,23	**0,74**	0,57	0,40	-0,01
DL1	-0,66	0,53	0,51	0,31	0,33	N/A	0,45
ML14	-0,29	-0,57	-0,20	**-0,86**	-0,53	N/A	-0,32
ML23	-0,07	-0,44	-0,14	**-0,78**	-0,42	N/A	-0,20
MH20	0,26	-0,52	-0,49	0,04	-0,07	0,59	-0,51
FL1	0,45	-0,04	0,10	0,31	-0,15	N/A	0,26
FH3	0,30	0,20	0,00	0,65	0,45	**0,74**	-0,08
FH5	0,25	-0,04	-0,01	-0,17	-0,04	-0,56	0,11
DL13	0,67	-0,21	-0,29	-0,21	0,27	0,70	-0,30
DH12	0,55	-0,49	-0,08	-0,41	-0,30	**0,78**	-0,04
TL1	0,22	-0,27	-0,10	-0,13	-0,25	-0,10	0,16
RA8	-0,30	-0,28	-0,43	-0,29	-0,38	0,67	0,11
MA5	0,25	-0,19	-0,65	-0,31	-0,13	N/A	-0,34
MA12	-0,52	0,28	0,11	0,37	0,28	0,17	0,18
MA13	-0,22	0,32	0,16	0,42	0,15	**-0,79**	0,30
MH1	0,02	0,26	-0,25	0,55	-0,03	0,18	0,35
MH2	-0,38	-0,42	**-0,74**	-0,16	-0,08	**-0,79**	-0,54
dmin	-0,57	**0,77**	0,46	**0,81**	0,65	N/A	0,57
dmax	0,34	-0,61	-0,55	-0,13	-0,04	N/A	-0,68
dD	0,14	0,43	0,16	0,50	0,26	N/A	0,54
dSD	0,08	0,46	0,17	0,51	0,30	N/A	0,54
dramp	-0,34	**-0,87**	-0,59	-0,57	-0,58	N/A	-0,57
dpath	0,12	0,42	0,16	0,45	0,21	N/A	0,55
drev	0,19	0,39	0,03	0,51	0,26	N/A	0,49
drf	0,67	0,65	0,52	0,10	0,39	N/A	0,65
dstD	0,10	0,34	0,09	0,27	0,11	N/A	0,52
dAstD	-0,31	0,08	-0,01	-0,08	-0,24	N/A	0,45
dCV	-0,32	0,15	0,04	-0,09	-0,16	N/A	0,47
dstMHramp	-0,25	-0,61	-0,22	**-0,71**	-0,54	N/A	-0,39
dflash	0,09	0,29	0,09	0,18	0,04	N/A	0,51

Annex II (cont.)

b)	EFI+						
Hydrological variable	Transect 1	Transect 2	Transect 3	Transect 4	Transect 5	Transect 6	Transect 2, 3 & 4
Depth	0,00	-0,04	0,10	0,69	0,58	0,51	0,13
Velocity	0,00	0,35	0,24	0,14	0,24	-0,34	0,63
MA3	0,00	0,50	0,06	-0,16	-0,26	0,41	-0,03
MA44	0,00	0,48	0,29	-0,45	-0,41	-0,52	-0,28
ML13	0,00	0,38	0,35	-0,35	-0,11	-0,67	0,29
DL1	0,00	-0,02	0,00	-0,32	0,08	N/A	-0,08
ML14	0,00	-0,13	0,03	0,46	0,13	N/A	-0,38
ML23	0,00	-0,14	0,07	0,34	0,05	N/A	-0,41
MH20	0,00	0,61	0,04	-0,20	-0,25	-0,17	0,01
FL1	0,00	0,58	-0,04	-0,03	0,14	N/A	0,41
FH3	0,00	0,34	0,02	-0,53	-0,35	-0,51	0,29
FH5	0,00	0,10	-0,20	-0,34	-0,02	0,65	-0,52
DL13	0,00	0,16	0,40	-0,25	-0,66	-0,57	-0,48
DH12	0,00	0,37	-0,12	-0,09	-0,36	-0,62	-0,60
TL1	0,00	0,51	-0,06	-0,30	-0,21	0,57	-0,20
RA8	0,00	0,43	0,22	0,23	0,39	-0,26	-0,22
MA5	0,00	-0,36	0,14	0,15	0,35	N/A	0,33
MA12	0,00	0,19	-0,01	-0,49	-0,11	**0,73**	0,11
MA13	0,00	0,24	0,02	-0,21	0,24	-0,11	0,30
MH1	0,00	0,20	0,01	0,23	0,52	**0,73**	0,48
MH2	0,00	0,52	0,55	-0,08	-0,01	-0,11	0,15
dmin	0,00	-0,02	0,08	-0,39	-0,18	N/A	0,29
dmax	0,00	0,47	0,12	-0,11	-0,38	N/A	-0,06
dD	0,00	0,36	0,14	-0,48	-0,08	N/A	0,06
dSD	0,00	0,34	0,13	-0,53	-0,11	N/A	0,03
dramp	0,00	0,48	0,12	0,50	0,16	N/A	0,02
dpath	0,00	0,33	0,15	-0,43	-0,03	N/A	0,09
drev	0,00	0,45	0,31	-0,38	-0,04	N/A	0,18
drf	0,00	-0,24	0,19	-0,07	0,08	N/A	-0,47
dstD	0,00	0,27	0,13	-0,43	-0,02	N/A	-0,07
dAstD	0,00	0,20	0,09	-0,18	0,06	N/A	-0,07
dCV	0,00	0,11	0,06	-0,29	0,00	N/A	-0,20
dstMHramp	0,00	-0,10	-0,06	0,60	0,17	N/A	-0,10
dflash	0,00	0,23	0,14	-0,37	0,00	N/A	-0,07

Annex III Correlations between the IBICAT2010 and the hydrological indices computed for 3, 6, 9, 24, 36 and 48 months of records, as well as for the hydrological year (values greater than 70% are in bold type; daily and subdaily indices are separated by double line). Hydrological indices (meaning in Table 2) are followed by a termination that indicates the period used. Indices not computed due to absent or constant flow records are marked as 'N/A'

Hydrological index	Transect 1	Transect 2	Transect 3	Transect 4	Transect 5	Transect 6	Transect 2, 3 & 4
MA3_3	-0,06	0,06	0,01	-0,07	0,23	-0,48	-0,14
MA44_3	-0,11	0,04	-0,06	-0,04	0,26	0,07	-0,11
ML13_3	-0,24	-0,07	-0,12	-0,33	0,11	N/A	-0,31
DL1_3	0,45	0,65	0,44	0,38	0,41	N/A	0,59
ML14_3	0,10	0,24	0,28	-0,05	-0,20	N/A	0,27
ML23_3	0,04	0,24	0,23	-0,15	-0,10	N/A	0,27
MH20_3	-0,19	-0,02	-0,10	-0,28	0,17	-0,09	-0,26
FL1_3	0,20	0,29	0,30	0,58	0,52	N/A	0,56
FH5_3	-0,24	0,50	0,44	0,56	0,49	-0,44	**0,72**
DL13_3	-0,25	0,38	0,40	0,01	0,06	N/A	0,40
DH12_3	-0,33	0,33	0,43	-0,13	0,08	0,17	0,21
TL1_3	-0,55	0,18	-0,01	-0,46	0,24	-0,48	-0,13
RA8_3	0,13	0,13	0,20	0,14	0,02	-0,49	0,17
MA5_3	-0,30	-0,26	-0,06	-0,07	-0,30	N/A	-0,22
MA12_3	-0,53	0,30	0,40	0,53	0,20	0,22	0,57
MA13_3	0,04	0,16	0,25	0,59	0,17	-0,23	0,46
MH1_3	0,58	-0,39	-0,34	0,51	0,14	0,22	-0,10
MH2_3	-0,40	0,06	0,20	-0,13	0,14	-0,23	0,07
MA3_6	-0,11	0,26	0,14	-0,33	0,15	-0,64	-0,11
MA44_6	0,01	0,29	0,22	-0,10	0,15	0,14	0,03
ML13_6	0,60	-0,12	-0,20	0,42	0,28	N/A	0,02
DL1_6	0,64	0,44	0,31	0,59	0,26	N/A	0,63
ML14_6	-0,33	0,09	0,18	-0,44	-0,33	N/A	-0,02
ML23_6	-0,22	-0,12	0,00	-0,21	-0,30	N/A	-0,09
MH20_6	0,07	0,24	0,11	-0,19	0,22	-0,11	-0,06
FL1_6	-0,24	0,41	0,38	0,66	0,53	N/A	0,65
FH5_6	-0,35	0,22	0,34	0,17	0,04	-0,66	0,26
DL13_6	-0,46	0,28	0,27	-0,30	-0,08	0,30	0,16
DH12_6	-0,45	0,29	0,37	-0,45	-0,13	0,22	-0,07
TL1_6	0,50	0,01	-0,07	-0,19	0,15	-0,48	-0,06
RA8_6	0,02	0,03	0,18	0,08	0,08	-0,04	0,09
MA5_6	N/A	-0,32	-0,14	-0,20	-0,27	N/A	-0,18
MA12_6	-0,53	0,30	0,40	0,53	0,20	0,22	0,57
MA13_6	0,04	0,16	0,25	0,59	0,17	-0,23	0,46
MH1_6	0,58	-0,39	-0,34	0,51	0,14	0,22	-0,10
MH2_6	-0,40	0,06	0,20	-0,13	0,14	-0,23	0,07

Annex III (cont.)

Hydrological index	Transect 1	Transect 2	Transect 3	Transect 4	Transect 5	Transect 6	Transect 2, 3 & 4
MA3_9	-0,55	0,40	0,37	-0,04	0,48	-0,64	0,21
MA44_9	-0,62	0,39	0,47	0,17	0,47	0,66	0,41
ML13_9	0,05	0,26	0,16	**0,77**	0,70	0,60	0,52
DL1_9	0,57	0,29	0,26	0,55	0,09	N/A	0,53
ML14_9	-0,54	-0,28	-0,19	**-0,87**	-0,48	-0,25	-0,55
ML23_9	-0,70	-0,21	-0,10	**-0,80**	-0,46	-0,25	-0,44
MH20_9	-0,20	0,51	0,40	0,24	0,69	**0,97**	0,37
FL1_9	-0,36	0,06	0,06	0,32	-0,05	N/A	0,13
FH5_9	-0,62	0,21	0,32	0,05	0,06	**-0,73**	0,25
DL13_9	**-0,74**	0,00	0,00	-0,68	-0,10	-0,08	-0,27
DH12_9	**-0,76**	0,20	0,30	-0,60	-0,04	0,56	-0,18
TL1_9	-0,28	0,43	0,68	-0,01	0,05	-0,48	0,40
RA8_9	0,33	-0,18	-0,02	-0,28	-0,14	0,48	-0,30
MA5_9	N/A	-0,52	-0,40	-0,06	-0,12	N/A	-0,31
MA12_9	-0,53	0,30	0,40	0,53	0,20	0,22	0,57
MA13_9	0,04	0,16	0,25	0,59	0,17	-0,23	0,46
MH1_9	0,58	-0,39	-0,34	0,51	0,14	0,22	-0,10
MH2_9	-0,40	0,06	0,20	-0,13	0,14	-0,23	0,07
MA3_year	-0,38	0,48	0,42	0,13	0,56	**-0,75**	0,32
MA44_year	-0,10	0,60	0,58	0,45	0,62	**0,71**	0,61
ML13_year	0,14	0,42	0,26	**0,90**	0,75	**0,90**	0,69
DL1_year	0,37	0,27	0,29	0,61	0,16	N/A	0,57
ML14_year	-0,29	-0,40	-0,33	**-0,88**	-0,63	N/A	-0,69
ML23_year	-0,54	-0,42	-0,31	-0,68	-0,53	N/A	-0,56
MH20_year	-0,20	0,51	0,40	0,24	0,69	**0,97**	0,37
FL1_year	-0,34	0,02	0,16	0,60	0,13	N/A	0,36
FH3_year	0,05	0,62	0,44	**0,75**	0,82	0,80	**0,75**
FH5_year	-0,47	-0,06	0,24	0,21	-0,19	-0,56	0,18
DL13_year	-0,25	0,29	0,11	-0,42	0,10	0,30	-0,02
DH12_year	-0,27	0,50	0,51	0,15	0,52	**0,75**	0,38
TL1_year	-0,70	0,20	0,55	0,05	-0,25	N/A	0,30
RA8_year	0,18	-0,24	0,13	-0,28	-0,43	**0,75**	-0,22
MA5_year	0,11	-0,66	-0,46	-0,19	-0,42	N/A	-0,53
MA12_year	-0,53	0,30	0,40	0,53	0,20	0,22	0,57
MA13_year	0,04	0,16	0,25	0,59	0,17	-0,17	0,46
MH1_year	0,58	-0,39	-0,34	0,51	0,14	0,22	-0,10
MH2_year	-0,40	0,06	0,20	-0,13	0,14	-0,23	0,07

Annex III (cont.)

Hydrological index	Transect 1	Transect 2	Transect 3	Transect 4	Transect 5	Transect 6	Transect 2, 3 & 4
MA3_24	-0,29	0,52	0,42	0,22	0,44	N/A	0,44
MA44_24	0,12	0,68	0,56	0,47	0,48	N/A	**0,71**
ML13_24	0,13	0,53	0,51	**0,75**	0,56	N/A	**0,79**
DL1_24	0,79	0,25	0,24	0,17	0,35	N/A	0,29
ML14_24	-0,09	-0,11	-0,20	**-0,72**	-0,35	N/A	-0,43
ML23_24	-0,43	-0,27	-0,31	-0,63	-0,52	N/A	-0,47
MH20_24	-0,45	0,39	0,47	0,30	0,32	N/A	0,41
FL1_24	-0,43	0,22	0,31	0,48	0,33	N/A	0,45
FH3_24	0,16	0,56	0,48	**0,85**	**0,76**	N/A	**0,79**
FH5_24	-0,26	-0,23	0,12	-0,06	-0,27	N/A	-0,13
DL13_24	-0,04	0,50	0,33	0,06	0,19	N/A	0,41
DH12_24	-0,36	0,70	0,68	0,06	0,29	N/A	0,50
TL1_24	0,02	-0,11	-0,14	-0,21	0,15	N/A	-0,30
RA8_24	0,06	-0,61	-0,31	-0,61	-0,58	N/A	-0,67
MA5_24	-0,70	-0,34	-0,10	0,02	-0,18	N/A	-0,11
MA12_24	0,08	-0,29	-0,23	0,32	0,07	N/A	-0,06
MA13_24	-0,01	-0,29	-0,15	0,20	0,05	N/A	-0,09
MH1_24	0,26	-0,34	0,09	-0,35	-0,64	N/A	-0,23
MH2_24	**-0,72**	-0,16	-0,20	-0,12	-0,15	N/A	-0,19
MA3_36	-0,22	0,66	0,43	0,47	0,62	N/A	0,59
MA44_36	-0,02	**0,86**	0,67	0,67	**0,72**	N/A	**0,89**
ML13_36	-0,04	0,57	0,44	0,68	0,55	N/A	**0,77**
DL1_36	0,65	0,33	0,38	-0,14	0,12	N/A	0,26
ML14_36	0,19	0,04	-0,18	-0,58	-0,16	N/A	-0,32
ML23_36	-0,02	-0,14	-0,31	-0,55	-0,23	N/A	-0,46
MH20_36	-0,57	-0,08	-0,12	0,42	0,33	N/A	0,04
FL1_36	0,10	-0,33	-0,06	-0,40	-0,36	N/A	-0,32
FH3_36	-0,04	0,53	0,58	**0,78**	0,62	N/A	**0,77**
FH5_36	-0,08	-0,21	0,13	-0,34	-0,23	N/A	-0,23
DL13_36	-0,02	**0,71**	0,39	0,35	0,55	N/A	0,62
DH12_36	0,20	0,45	0,43	-0,10	0,09	N/A	0,17
TL1_36	-0,36	0,02	0,04	-0,44	-0,09	N/A	-0,23
RA8_36	0,44	-0,63	-0,46	**-0,86**	-0,67	N/A	**-0,86**
MA5_36	**-0,72**	-0,38	-0,18	-0,15	-0,29	N/A	-0,24
MA12_36	**0,73**	-0,13	0,14	-0,45	-0,40	N/A	-0,24
MA13_36	0,36	-0,01	0,21	-0,56	-0,42	N/A	-0,27
MH1_36	-0,13	-0,45	-0,61	-0,59	-0,45	N/A	-0,60
MH2_36	0,63	-0,12	-0,02	0,21	-0,23	N/A	-0,06

Annex III (cont.)

Hydrological index	Transect 1	Transect 2	Transect 3	Transect 4	Transect 5	Transect 6	Transect 2, 3 & 4
MA3_48	N/A	**0,72**	0,69	0,61	0,65	N/A	**0,73**
MA44_48	N/A	0,66	0,54	0,42	0,66	N/A	0,65
ML13_48	N/A	0,53	0,40	0,59	0,59	N/A	0,70
DL1_48	N/A	0,39	0,20	-0,12	0,31	N/A	0,18
ML14_48	N/A	0,22	-0,11	-0,35	0,09	N/A	-0,10
ML23_48	N/A	-0,18	-0,33	-0,62	-0,35	N/A	-0,49
MH20_48	N/A	-0,11	0,26	-0,15	-0,26	N/A	-0,17
FL1_48	N/A	-0,33	-0,06	-0,40	-0,36	N/A	-0,32
FH3_48	N/A	0,42	0,65	0,57	0,37	N/A	0,57
FH5_48	N/A	-0,21	0,13	-0,34	-0,23	N/A	-0,23
DL13_48	N/A	0,74	0,47	0,42	0,68	N/A	0,72
DH12_48	N/A	0,38	0,40	-0,16	0,08	N/A	0,11
TL1_48	N/A	-0,09	-0,27	-0,51	-0,16	N/A	-0,47
RA8_48	N/A	-0,55	-0,40	-0,92	**-0,73**	N/A	-0,83
MA5_48	N/A	-0,22	0,03	-0,16	-0,22	N/A	-0,15
MA12_48	N/A	-0,01	-0,14	**-0,77**	-0,22	N/A	-0,41
MA13_48	N/A	-0,22	-0,39	-0,55	-0,07	N/A	-0,46
MH1_48	N/A	0,55	0,34	0,05	0,31	N/A	0,36
MH2_48	N/A	-0,44	-0,36	-0,08	-0,08	N/A	-0,34
dmin_1	0,70	0,24	0,18	0,48	0,14	N/A	0,35
dmax_1	0,27	0,17	-0,02	0,31	0,49	N/A	0,20
dD_1	0,51	0,02	-0,16	0,55	0,57	N/A	0,19
dSD_1	0,54	-0,02	-0,18	0,54	0,56	N/A	0,16
dramp_1	0,21	0,16	0,00	0,30	0,43	N/A	0,22
dpath_1	0,53	0,05	-0,12	0,60	0,58	N/A	0,24
drev_1	0,35	0,14	-0,03	0,58	0,63	N/A	0,33
drf_1	0,38	-0,17	-0,07	0,15	-0,21	N/A	0,04
dstD_1	0,21	0,20	0,02	0,67	0,70	N/A	0,41
dAstD_1	0,21	0,21	0,03	**0,71**	**0,71**	N/A	0,43
dCV_1	0,27	0,16	-0,02	0,70	0,69	N/A	0,39
dstMHramp_1	0,09	0,28	0,12	0,38	0,47	N/A	0,36
dflash_1	0,17	0,23	0,06	**0,73**	0,70	N/A	0,46

Annex III (cont.)

Hydrological index	Transect 1	Transect 2	Transect 3	Transect 4	Transect 5	Transect 6	Transect 2, 3 & 4
dmin_3	0,42	-0,14	-0,21	0,44	0,04	N/A	0,60
dmax_3	-0,12	-0,08	-0,13	-0,21	0,14	N/A	-0,12
dD_3	0,19	-0,07	-0,15	0,41	0,39	N/A	0,04
dSD_3	0,22	-0,07	-0,15	0,42	0,37	N/A	0,03
dramp_3	0,04	0,15	0,11	-0,02	0,27	N/A	0,06
dpath_3	0,21	0,01	-0,10	0,56	0,50	N/A	0,14
drev_3	0,29	0,25	0,08	**0,80**	**0,72**	N/A	0,34
drf_3	0,15	0,09	0,20	0,15	-0,05	N/A	-0,01
dstD_3	0,18	0,33	0,21	**0,86**	**0,73**	N/A	0,34
dAstD_3	0,14	0,53	0,44	**0,86**	**0,74**	N/A	0,55
dCV_3	0,22	0,49	0,41	**0,82**	0,69	N/A	0,53
dstMHramp_3	0,13	0,42	0,42	0,06	0,25	N/A	0,20
dflash_3	0,14	0,43	0,33	**0,92**	**0,74**	N/A	0,42
dmin_6	**0,74**	-0,13	-0,22	0,44	0,03	N/A	0,61
dmax_6	0,01	0,13	-0,06	-0,19	0,20	N/A	-0,14
dD_6	0,18	0,24	0,16	0,65	0,49	N/A	0,35
dSD_6	0,19	0,21	0,15	0,64	0,46	N/A	0,33
dramp_6	-0,15	0,02	-0,25	-0,26	0,20	N/A	-0,23
dpath_6	0,13	0,29	0,24	**0,71**	0,54	N/A	0,45
drev_6	0,16	0,41	0,34	**0,84**	0,68	N/A	0,57
drf_6	0,11	-0,07	-0,07	0,29	0,01	N/A	0,01
dstD_6	-0,14	0,43	0,38	0,63	0,61	N/A	0,44
dAstD_6	-0,36	0,49	0,45	0,46	0,54	N/A	0,46
dCV_6	-0,37	0,46	0,43	0,43	0,50	N/A	0,32
dstMHramp_6	-0,40	0,01	-0,24	-0,38	0,01	N/A	-0,30
dflash_6	-0,31	0,47	0,46	0,53	0,57	N/A	0,54
dmin_9	0,66	0,00	-0,08	0,39	-0,08	N/A	0,57
dmax_9	-0,31	0,35	0,27	0,23	0,63	N/A	0,18
dD_9	0,32	0,32	0,43	**0,77**	0,41	N/A	0,54
dSD_9	0,35	0,27	0,41	**0,72**	0,32	N/A	0,57
dramp_9	-0,34	-0,11	-0,27	-0,27	0,26	N/A	-0,37
dpath_9	0,28	0,32	0,50	**0,73**	0,33	N/A	0,50
drev_9	0,35	0,35	0,50	**0,75**	0,35	N/A	0,55
drf_9	0,27	-0,01	0,05	0,37	-0,04	N/A	-0,01
dstD_9	0,02	0,27	0,42	0,52	0,36	N/A	0,31
dAstD_9	0,27	-0,09	0,24	-0,09	-0,27	N/A	-0,07
dCV_9	0,35	-0,14	0,18	-0,13	-0,31	N/A	-0,02
dstMHramp_9	-0,33	-0,39	-0,54	**-0,74**	-0,33	N/A	-0,64
dflash_9	-0,18	0,19	0,41	0,30	0,21	N/A	0,25

Annex III (cont.)

Hydrological index	Transect 1	Transect 2	Transect 3	Transect 4	Transect 5	Transect 6	Transect 2, 3 & 4
dmin_year	0,43	-0,35	-0,35	0,22	-0,25	N/A	0,60
dmax_year	-0,31	0,35	0,27	0,23	0,63	N/A	0,16
dD_year	0,15	0,38	0,59	0,63	0,34	N/A	0,63
dSD_year	0,17	0,35	0,58	0,59	0,28	N/A	0,66
dramp_year	-0,12	-0,38	-0,24	-0,67	-0,24	N/A	-0,47
dpath_year	0,13	0,35	0,59	0,54	0,26	N/A	0,56
drev_year	0,18	0,39	0,57	0,58	0,33	N/A	0,55
drf_year	0,38	0,44	0,37	0,54	0,27	N/A	0,03
dstD_year	-0,03	0,26	0,55	0,37	0,20	N/A	0,41
dAstD_year	-0,02	-0,02	0,36	0,21	-0,06	N/A	0,26
dCV_year	0,02	-0,05	0,35	0,17	-0,11	N/A	0,30
dstMHramp_year	0,03	-0,31	0,11	-0,24	-0,31	N/A	-0,14
dflash_year	-0,10	0,21	0,50	0,19	0,08	N/A	0,33
dmin_24	0,74	0,05	0,03	0,52	0,17	N/A	0,38
dmax_24	-0,50	0,30	0,27	0,16	0,24	N/A	0,27
dD_24	-0,09	0,05	0,26	0,32	0,14	N/A	0,28
dSD_24	-0,08	0,07	0,27	0,36	0,16	N/A	0,31
dramp_24	-0,09	0,10	0,06	-0,32	-0,10	N/A	-0,15
dpath_24	-0,14	0,00	0,21	0,28	0,08	N/A	0,23
drev_24	-0,07	-0,01	0,19	0,31	0,07	N/A	0,25
drf_24	0,33	0,03	-0,04	-0,02	0,16	N/A	0,02
dstD_24	-0,29	-0,18	0,10	0,01	-0,14	N/A	-0,03
dAstD_24	-0,20	-0,50	-0,24	-0,55	-0,58	N/A	-0,55
dCV_24	-0,13	-0,49	-0,25	-0,56	-0,55	N/A	-0,56
dstMHramp_24	-0,20	-0,34	-0,40	-0,79	-0,50	N/A	-0,67
dflash_24	-0,41	-0,22	0,05	-0,03	-0,19	N/A	-0,06
dmin_36	0,61	0,21	0,17	0,28	0,18	N/A	0,40
dmax_36	-0,53	-0,05	-0,09	0,44	0,34	N/A	0,06
dD_36	-0,22	0,04	0,33	-0,05	-0,06	N/A	0,11
dSD_36	-0,17	0,06	0,34	0,01	-0,02	N/A	0,16
dramp_36	0,07	-0,25	-0,32	0,00	0,05	N/A	-0,34
dpath_36	-0,23	0,02	0,30	-0,07	-0,10	N/A	0,11
drev_36	-0,23	0,03	0,27	0,03	-0,08	N/A	0,18
drf_36	-0,15	0,35	0,31	-0,25	0,11	N/A	0,20
dstD_36	-0,45	-0,16	0,15	-0,37	-0,29	N/A	-0,18
dAstD_36	-0,06	-0,47	-0,27	-0,87	-0,67	N/A	-0,70
dCV_36	-0,07	-0,47	-0,31	-0,88	-0,68	N/A	-0,69
dstMHramp_36	-0,01	-0,40	-0,53	-0,46	-0,26	N/A	-0,63
dflash_36	-0,57	-0,15	0,14	-0,40	-0,33	N/A	-0,16

Annex III (cont.)

Hydrological index	Transect 1	Transect 2	Transect 3	Transect 4	Transect 5	Transect 6	Transect 2, 3 & 4
dmin_48	N/A	0,42	0,23	0,26	0,32	N/A	0,50
dmax_48	N/A	-0,07	0,29	-0,15	-0,25	N/A	-0,14
dD_48	N/A	0,15	0,32	-0,40	-0,10	N/A	-0,07
dSD_48	N/A	0,17	0,36	-0,34	-0,07	N/A	-0,01
dramp_48	N/A	-0,31	0,00	-0,42	-0,47	N/A	-0,46
dpath_48	N/A	0,17	0,33	-0,38	-0,10	N/A	-0,02
drev_48	N/A	0,23	0,35	-0,20	-0,01	N/A	0,15
drf_48	N/A	0,43	0,18	-0,05	0,39	N/A	0,24
dstD_48	N/A	0,02	0,19	-0,69	-0,27	N/A	-0,27
dAstD_48	N/A	-0,23	-0,08	-0,90	-0,58	N/A	-0,58
dCV_48	N/A	-0,17	0,02	-0,84	-0,47	N/A	-0,47
dstMHramp_48	N/A	-0,44	-0,27	-0,52	-0,58	N/A	-0,60
dflash_48	N/A	0,04	0,20	-0,64	-0,29	N/A	-0,21

Chapter 3. Normalized Abundance Spectra of fish community reflect hydro-peaking and high presence of alien species on a Mediterranean large River

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Abstract

The European Water Framework Directive requires the integration of body size characters as an important part of fish-based bioassessment tools for freshwaters ecological status determination. The study of the entire fish community size-structure provides valuable information about food web capacity, food web stability and ecological efficiency of aquatic ecosystems. One of the most used representations of community size structure is the Normalized Abundance Spectra (NAS) that provides an approximation of the total fish abundance and food web capacity (through the parameter y-intercept) and an estimation of food web efficiency (through the slope of the linear regression). In this study we explored NAS of the lower Ebro River fish community by integrating data from monthly electrofishing samplings during a whole year (November 2014- October 2015). We found that the percentage of total alien and alien-prey individuals were directly related with y-intercept and inversely related with slope of NAS. This is because the bulk of the community consists of introduced species of small body length. Furthermore, we detected significant relationships between NAS-related parameters and the hydrological variables describing diel flow oscillations and daily flow variability. Based on this, we suggest that high flow variability conditions and, above all, high hydro-peaking conditions, caused a diminution of the total abundance of fish and a decrease of the proportion of small sized fish (i.e. lower y-intercept and flatter slopes of NAS, respectively). Finally, a significant interaction between hydro-peaking and the percentage of alien-predators suggests that high hydro-peaking conditions benefit predation by facilitating predator-prey encounters. This is reflected by strong linear relationship between NAS parameters and percentage of piscivorous at high hydropeaking conditions that disappears at low hydro-peaking fluctuations. We concluded that the high proportion of alien fishes and the presence of a hydropower generation plant that operates by hydro-peaking are important factors determining fish size structure in the lower Ebro River.

Key words

Size Structure, fish community, Ebro River, hydro-peaking, alien species, predatory pressure

Highlights

1- Small sized species of alien fish dominate the lower Ebro River fish community.
2- Normalized Abundance Spectra (NAS) of fish were evaluated monthly for one year.
3- NAS reflected hydro-peaking impacts and the high presence of alien species.
4- High hydro-peaking conditions may increase predatory efficiency of piscivorous fish.

Introduction

Since the implementation of the European Water Framework Directive (WFD) (European Community, 2000) a huge effort has been done to find suitable bioassessment tools for the assessment of freshwater ecosystems health. Indeed, the WFD requires the use of size characteristics as a normative condition for fish-based bioassessment tools (Murphy et al., 2013; Reyjol et al., 2014), and it is certainly true that size structures of aquatic communities are shaped by biotic interactions and environmental factors, and may therefore reflect changes occurred on the surrounding conditions, whether of anthropogenic or natural origin (Blanchard et al., 2017; Emmrich et al., 2014; Murry and Farrell, 2014; Sprules and Barth, 2016). There are many approaches to visualize the size distribution of organisms in a sample that have become increasingly popular since the formulation of the biomass size spectrum theory in the mid-1960s. This theory states that in aquatic ecosystems, the sum of biomass is approximately constant across equal logarithmic intervals of body size from the smallest to the biggest organism (Sprules and Barth, 2016). From this idea emerged the Normalized Abundance Spectra (NAS) that can be adjusted to a linear model and provide information about food-web capacity (through the y-intercept of the spectrum) and ecological efficiency of communities (through the slope of the spectrum) (Daan et al., 2005; Mehner et al., in press; Rice and Gislason, 1996). Size structure approaches have been extensively used to study the ecosystems in marine environments (Andersen and Beyer, 2006; Blanchard et al., 2017, 2005; Kimmel et al., 2006; Platt and Denman, 1977; Sheldon et al., 1972), and in lakes and lagoons (Arranz et al., 2015; Brucet et al., 2010, 2005, Emmrich et al., 2014, 2011). But there are very few studies on rivers (Benejam et al., 2018, 2015; Broadway et al., 2015; Murry and Farrell, 2014). In this study we analysed Normalized Abundance Spectra (NAS) of the fish community of the lower Ebro River, a Mediterranean large river in the Iberian Peninsula, to test their ability to detect environmental and biotic disturbances.

The Ebro River is affected by several human impacts: water abstraction, dam regulation with subsequent altered hydrological regime (Batalla et al., 2004) and low suspended sediments (Rovira et al., 2007), industrial pollution (Benejam et al., 2010; Huertas et al., 2016), thermal pollution (Prats et al., 2012, 2010), proliferation of macrophytes (Ibáñez et al., 2012) and introduction of many alien fish species (Caiola et al., 2014; Elvira, 1995a, 1995b), among others. It is well known that non-native fish introductions can result in local decline and even extinction of native species through different mechanisms such as genetic introgression, introduction of parasites and diseases, competition for habitat, habitat alteration and predation (García-Berthou, 2007). Specifically in the lower Ebro River, where this study is focused, some of the new introduced species are voracious piscivorous (e.g. *Silurus glanis*) that can cause critical changes in the original fish populations because many indigenous species have evolved in communities with none native piscivorous fishes (Carol et al., 2009; Ribeiro and

Leunda, 2012). Another important source of alteration from the natural ecological status in the lower Ebro River is the presence of a hydroelectric generation power plant that operates by hydro-peaking. This is the procedure by which hydroelectricity is produced to cover the daily fluctuating demands of the energy market and it results on a daily peaking flow regulation that affects the biological dynamics of downstream communities. Many authors reported adverse impacts of hydro-peaking on fish caused by, inter alia, stranding, habitat abandonment, downstream displacement, inhibition of spawning, migration obstruction, habitat modification, increase of physiological stress and depleted food production (Boavida et al., 2015; Rui et al., 2017; Schmutz et al., 2015; Young et al., 2011). Among all the mentioned environmental disturbances, we looked for potential causes of alteration on NAS through an extensive sampling of fish stocks by electrofishing.

The main objective of the present paper is to identify the natural and anthropogenic factors affecting size structure of fish community in a large Mediterranean river. In this way, we want to evaluate the potential of Normalized Abundance Spectrum parameters as size-related bioindicators to complement the fish-based indices currently in use.

Material and methods

Study area

The Ebro River is located in the NE of the Iberian Peninsula (Fig. 1a). With a total drainage area of 85.569 km^2 and a mean annual flow of 294 m^3·s^{-1}, provides valuable ecosystem services to people such as water for agriculture uses (irrigation area of 906.000 ha), urban supply (basin population of 3.176.091) and hydro-power generation (458 stations) (Confederación Hidrográfica del Ebro, 2009).

In terms of hydrology the study area can be divided in two regions: the river section upstream from Xerta weir that comprises the stations E05 to E02 where the mean annual flow is of approximately 320 m^3·s^{-1}, and the reach after Xerta weir that comprises sampling station E01. This weir is the last human obstacle before the river mouth and its function is to divert water for agricultural uses and human consumption. The mean annual flow in station E01 is about 280m^3·s^{-1}. Hydro-peaking oscillations generated in a hydroelectrical power plant located in Flix dam are perceptible in all the study area (Figs. 1 and 2).

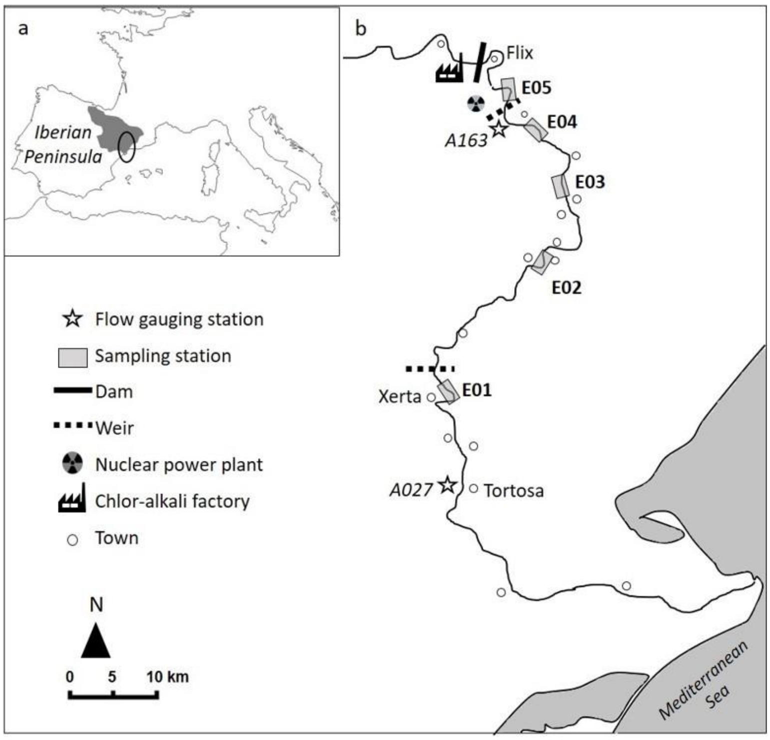

Figure 1. (a) Location of the Ebro River and (b) the five sampling stations.

Sampling

Data of fish species abundance and body size were collected by electro-fishing in 5 stations on the lower Ebro River (Fig. 1). The stations were randomly selected to cover all the hydromorphological variability of the study area (for more details see Caiola et al., 2014). Each station consisted in a 2 km length stretch to fulfil the requirements of EN 14011 European standard (CEN. European Committee for Standardization, 2003) that establishes a minimum sampling stretch length of ten times the river width (in the study area the mean width is *ca*. 180 m). Ten equidistant points located in the littoral zone were sampled within each station, randomly alternating left and right banks, with a total length sampled at each sampling station of about 200m. A boat based electro-fishing gear (Model: EL63IIGI, HANS GRASSL GmbH, Schönau am Königssee, Germany) was used at 600 V and 10 A pulsed D.C. The fish were collected with a dip net of 2.5 m long, 47 cm of diameter and 7 mm of mesh size. The specimens were identified to species level, counted, measured and weighted. Native specimens were returned to the river and alien fishes were sacrificed with an overdose of anesthetic (MS-222). We normalized abundances dividing by fished area and time of fishing, obtaining catches per unit effort (CPUE: individuals·ha^{-1}·min^{-1}) and biomass per unit effort (BPUE: kg·ha^{-1}·min^{-1}).

In addition to fish community metrics, we measured several environmental factors and habitat variables: water temperature (°C), conductivity (mS·cm^{-1}), dissolved oxygen (mg·l^{-1}) and total dissolved solids (mg·l^{-1}) were measured with a multi-parameter probe (YSI model 556 MPS); water depth (m) was measured using a portable depth-meter (Hondex model PS-7) and water flow velocity (m·s^{-1}) was measured with a current-meter (Global Water model FP101). The presence, absence and area covered by macrophytes was recorded by visual approximation following the recommendations for macrophytes assessment of the STAR project (Dawson, 2002). Daily and diel flow data series available at the Ebro Water Authority (CHE) web site (http://www.chebro.es/) were used to calculate hydrological indices (see section 2.5. Hydrological variables calculation).

Monthly sampling campaigns were conducted between November 2014 and October 2015. Due to safety questions, we avoided sampling on December 2014, February and March 2015, when the water flow at the study area was higher than 500 m^3·s^{-1} and it was impossible to guarantee the safety of the crew.

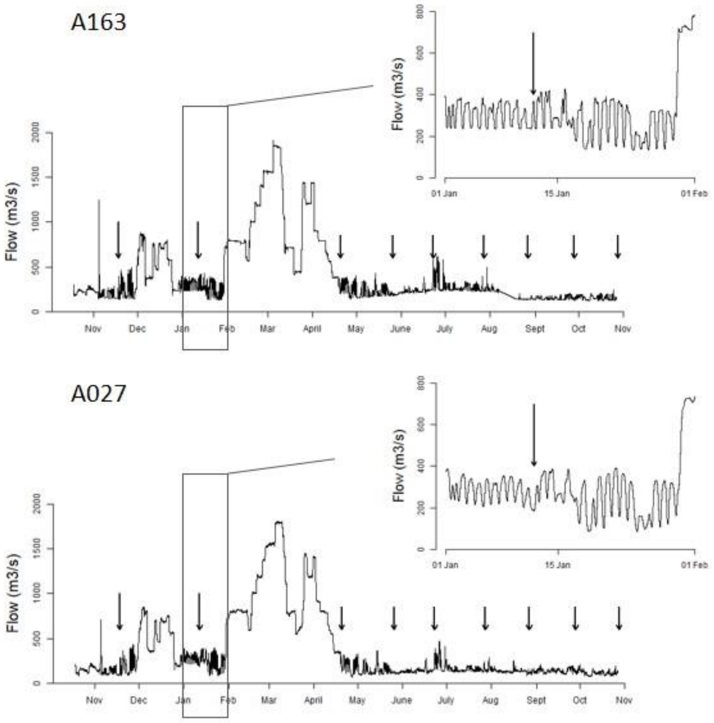

Figure 2. Hydrographs on gauging station A163 (upper figure) and on gauging station A027 (lower figure) during the study period November 2014 – October 2015. Arrows indicate sampling dates. Note the hydropeaking oscillations.

Fish size spectrum calculation

For each station and sampling period we modeled the Normalized Abundance Spectrum (NAS) with body lengths of the entire fish community. NAS is obtained through a binning method in which body size measurements are grouped into logarithmic size intervals and the abundance of individuals is represented for each size class in a $\log_2$-$\log_2$ plot. We standardized abundance by dividing it by the linear width of the size interval as described on Sprules and Barth (2016). The linear size spectra was calculated from NAS as the linear regression between the $\log_2$ midpoint of size classes (abscissas) and the $\log_2$ of normalized abundance per size class (ordinates) (Fig. 3). We considered six size classes following a $\log_2$ scale (1st class, $\leq 2^6=64$ mm; 2nd class, $>2^6=64$ mm to $2^7=128$ mm; 3rd class, $>2^7=128$ mm to $2^8=256$ mm; 4th class, $>2^8=256$ mm to $2^9=512$ mm; 5th class, $>2^9=512$ mm to $2^{10}=1024$ mm; 6th class, $>2^{10}=1024$ mm to maximum length). Electro-fishing has been shown as a suitable method to estimate size structure of fish in rivers (Benejam et al., 2016). However, although it has been demonstrated to be less selective than other sampling methods, it can imply an underestimation of small fish in some species due to the fact that the electric field has greater effects on large fish (Barbour et al., 1999; Borgstrøm and Skaala, 1993; Millar et al., 2016). For this reason, the smallest size classes were grouped in the first class (<64mm) (Benejam et al., 2016). We estimated the intercept and the slope for each linear size spectrum. The y-intercept of the linear size spectrum can be interpreted as an approximation of food web capacity (Gaedke and Straile, 1994; Murry and Farrell, 2014) whereas the slope equals to a measure of energy transfer or ecological efficiency (Emmrich et al., 2011, Mehner et al. in press). We only considered for the analysis the size distributions that fit a linear spectrum, so we discarded 3 regressions with p values >0.1 (Arranz et al., 2015) from a total of 45 (5 stations per 9 sampling campaigns).

Hydrological variables calculation

There are two official gauging points for water flow measurement in the study area: the gauging stations A163 in Ascó and A027 in Tortosa (Fig. 1). They belong to the Automatic System for Hydrological Information (SAIH) of the Ebro River (Confederación Hidrográfica del Ebro, 2002) and provide a flow measurement every 15 minutes. We used the A027 series as an approximation of the flow on sampling station E01 and the A163 series for the sampling stations E02, E03, E04 and E05.

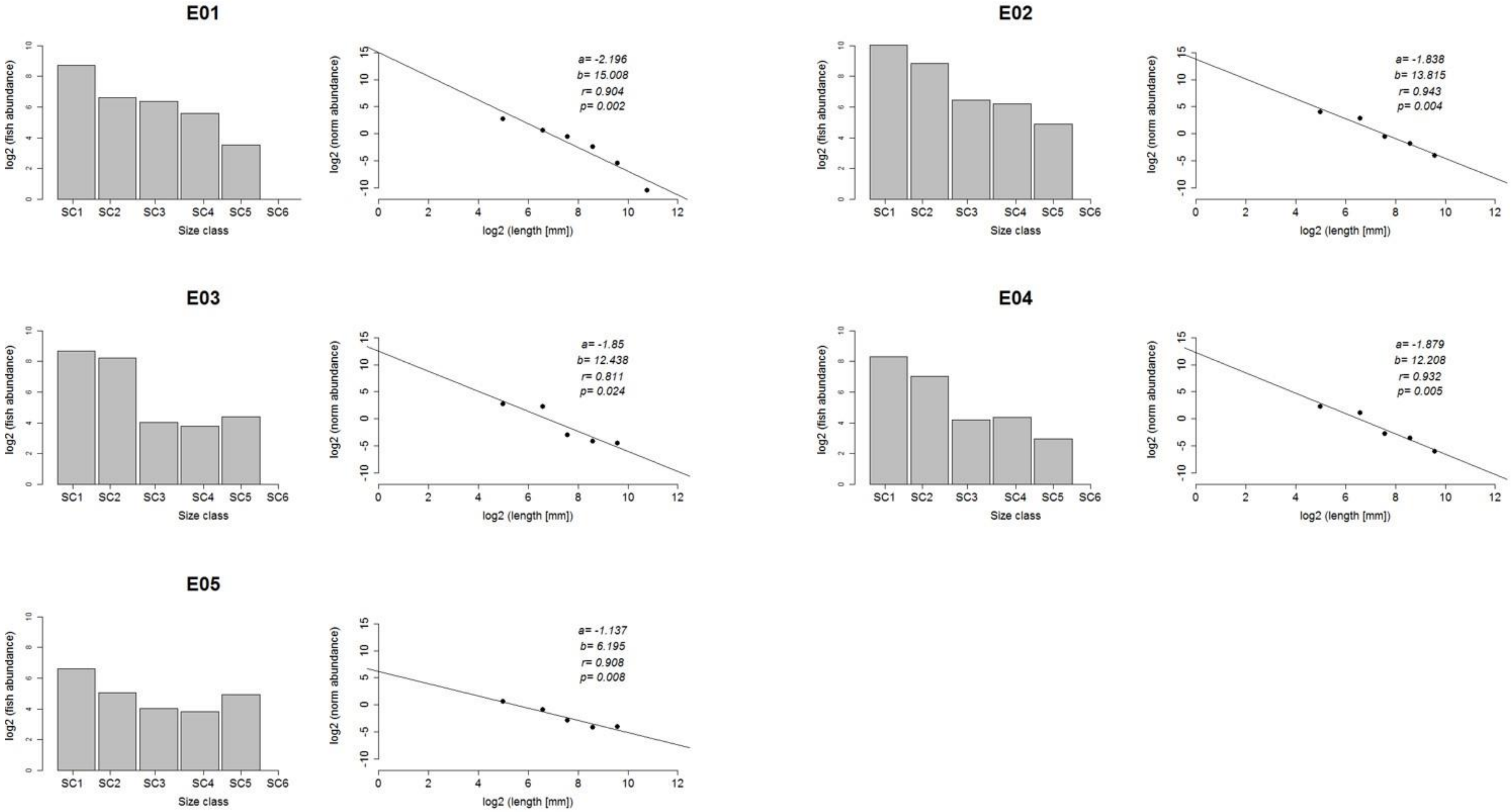

Figure 3. Examples of Abundance Frequency Distributions and Normalized Abundance Spectrum: Sampling campaign of April 2015; sites E01-E05. The linear parameters a (slope) and b (y-intercept) are shown, as well as their r and p values.

Index	**Calculation**
Daily indices	
M_A 3	Coefficient of variation in daily flows
M_A 44	Variability in daily flows divided by median monthly flow, where variability is calculated as 90th-10th percentile
M_L 13	Coefficient of variation in minimum flows
M_L 14	Lowest monthly flow divided by median monthly flow
M_H 17	25th percentile from the flow duration curve divided by median daily flow
M_H 20	Monthly maximum flow
F_L1	Number of occurrences during which the magnitude of flow remains below the 25th percentile
F_H5	Number of occurrences during which the magnitude of flow remains above the median daily flow
D_H12	Mean 7-day maximum divided by median flow
R_A8	Number of negative and positive changes in water conditions from one day to the next
M_A5	Skewness in daily flows: Mean daily flows divided by median daily flows
Subdaily indices	
dmin	Daily minimum
dmax	Daily maximum
dD	Daily delta or range
dSD	Daily Standard deviation
dramp	Maximum hourly ramp rate
dpath	Daily path length (the geometric distance of the daily hydrograph of flow versus time
drev	Number of changes between rising and falling periods)
drf	Rise and fall counts difference
dAstD	Annually standardized delta (monthly mean of daily delta divided by annual mean)
dCV	Coefficient of variation (daily standard deviation divided by the mean monthly daily flow)
dstMHramp	Standardized maximum hourly ramping rate (maximum daily ramp rate divided by the mean monthly daily flow
dflash	Richards-Baker flashiness index (daily path length of oscillations divided by the daily mean over each 24-h period)

Table 1 – Hydrological indices adapted from Olden & Poof (2003) and Bevelhimer et al (2014) for a period of one month before date of sampling. Daily indices were calculated from a daily data series and sub-daily indices from an hourly data series.

A collection of hydrological indices adapted from literature was calculated (Table 1). The set of daily indices were described in Olden and Poff (2003) as key variables to characterize the hydrological regime for our type of river (with snow and rain influence) while avoiding redundancy. We included adapted descriptors of magnitude of flow events (M_A3, M_A44, M_L13, M_L14, M_H17 and M_H20), frequency of flow events (F_L1 and F_H5), duration of flow events (D_H12) and the rate of change in flow conditions (R_A8). We also included the skewness in daily flows (M_A5), described by Kinsolving and Bain (1993) as important in the response of fish to anthropogenic flow alterations. Sub-daily indices were proposed by Bevelhimer et al. (2014) as good descriptors of diel flow variations such as the produced by power generation plants. Among them, there are two descriptors of magnitude (dmin and dmax) and one descriptor of the frequency of oscillations (drev). The rest are different ways of expressing the amplitude of flow fluctuations (dD, dSD, dramp, dpath, drf, dAstD, dCV, dstMHramp, dflash). We adapted all the indices to assess flow variations in a short temporal scale and calculated them for the period of one month before each sampling.

Biotic factors

To assess how the presence of alien species affects the size structure of the community, three biotic factors were considered: percentage of alien individuals, percentage of alien-piscivorous individuals (over total catches) and percentage of alien-prey individuals (over total catches). It should be noted that the only native piscivorous fish that we found was the sea bass (*Dicentrarchus labrax*), a marine species that occasionally migrate between freshwater and the sea (in both directions), for feeding purposes. We only fished 12 individuals of this species, always in the transect E01 (the station furthest downstream), so we could say that the piscivorous population is almost entirely constituted by alien fish (Table 2).

Species	**Common name**	**Feeding**	**Status**	**Total**	**CPUE**	**BPUE**
Anguilla anguilla	Eel	Omnivorous[1]	N	1879	321.2	$75.45\cdot10^3$
Dicentrarchus labrax	Seabass	Piscivorous[4]	N	12	1.7	$0.88\cdot10^3$
Gobio lozanoi	Iberian gudgeon	Invertivorous[2]	N	332	54.6	$0.11\cdot10^3$
Undetermined Mullet	Mullet	Detritivorous[4]	N	398	56.1	$8.14\cdot10^3$
Luciobarbus graellsii	Ebro barbel	Omnivorous[4]	N	85	10.9	$2.72\cdot10^3$
Salaria fluviatilis	Freshwater blenny	Invertivorous[2,3]	N	746	200.9	$1.49\cdot10^3$
Squalius laietanus	Catalan chub	Omnivorous[2]	N	1371	203.1	$2.95\cdot10^3$
Alburnus alburnus	Bleak	Omnivorous[2]	A	18567	2661.9	$113.95\cdot10^3$
Carassius auratus	Goldfish	Omnivorous[2,3]	A	174	24.5	$10.49\cdot10^3$
Cyprinus carpio	Common carp	Omnivorous[1,2,3]	A	343	48.4	$188.34\cdot10^3$
Gambusia holbrooki	Eastern mosquitofish	Invertivorous[2,3]	A	1448	273.8	$0.33\cdot10^3$
Lepomis gibbosus	Sunfish	Invertivorous[1,2,3]	A	113	15.0	$0.44\cdot10^3$
Micropterus salmoides	Largemouth black bass	Piscivorous[1,2,3]	A	1	0.1	$0.04\cdot10^2$
Pseudorasbora parva	Razbora	Omnivorous[2]	A	1118	157.7	$0.82\cdot10^3$
Perca fluviatilis	European perch	Piscivorous[2]	A	35	4.9	$0.16\cdot10^3$
Rutilus rutilus	Roach	Omnivorous[2]	A	528	74.5	$4.48\cdot10^3$
Scardinius erythrophthalmus	Rudd	Omnivorous[2]	A	79	11.1	$0.60\cdot10^3$
Silurus glanis	Wels catfish	Piscivorous[2]	A	213	30.0	$101.37\cdot10^3$
Sander lucioperca	Zander	Piscivorous[4]	A	203	28.6	$2.37\cdot10^3$

Table 2 – Latin names, common names, feeding behaviour, status refereed to the Ebro River (N=native; A=alien), total catches, mean CPUEs and mean BPUEs ([1]Alexandre et al., 2013; [2]García-Berthou et al., 2015; [3]Magalhães et al., 2008; [4]Ordeix et al., 2014)

To calculate the number of piscivorous, juvenile individuals considered too young to feed on fish, were subtracted for each predator species. That is: only individuals greater than 120 mm were considered piscivorous for *Perca fluviatilis* (Kottelat and Freyhof, 2007) and *Sander lucioperca* (Aparicio et al., 2016), while this threshold was 50 mm for *Silurus glanis* (Copp et al., 2009) and *Micropterus salmoides* (García-Berthou, 2002). To determine the percentage of alien-prey individuals, all the non-piscivorous alien fishes were considered susceptible to predation, so we calculated it as the subtraction of percentage of alien minus percentage of alien-piscivorous.

Statistical Analyses

To check for relationships between the response variables y-intercept of NAS (food web capacity) and slope of NAS (food web efficiency) and the potential explanatory variables (macrophyte coverage, water temperature, conductivity, dissolved oxygen, total dissolved solids, water depth, water velocity, percentage of alien fish, percentage of alien-piscivorous, percentage of alien-prey and the hydrological indices on Table 1), we performed simple lineal regressions with autocorrelation structures for time and space when necessary. The large number of explanatory variables that we wanted to include in the analysis prevented us from applying multiple regressions and thus, we performed simple lineal regressions in this first step. As our sampling design consists of several observations along time within each river stretch, it results on a data set with a possible lack of independence, both spatial and temporal. To deal with this limitation we used Mixed Effects Models with river reach as random factor and tested the inclusion of temporal auto-correlation structures to improve the models as described in Zuur et al. (2009). Firstly, we tested 5 types of auto correlation structures: AR-1, linear correlation, Gaussian correlation, exponential correlation and spherical correlation. Once we knew the best temporal auto-correlation expression we proceeded to choose the best model comparing: a) a linear regression model without any autocorrelation; b) a linear regression model with the best autocorrelation structure for time; c) a mixed effects model with river reach as random factor and d) a mixed effects model with river reach as random factor and the best autocorrelation structure for time. The models were adjusted using the R package nlme (Pinheiro et al., 2017; R Core Team, 2016) and compared with the Akaike Information Criterion (AIC) (Akaike, 1974). The most parsimonious model (with the lowest AIC) was selected each time.

In a second step, from the initial set of hydrological variables, we considered only those that were significantly related with size spectra parameters in the previous analysis to investigate possible multiplicative effects between hydrology and biotic factors. To avoid redundant tests, we first calculated the correlation matrix for all factors and discarded correlated hydrological indices with $r > 0.6$. Then, interactions were tested for all the possible combinations between the three biotic factors (percentage of alien, percentage of alien-prey and percentage of alien-piscivorous) and the three uncorrelated hydrological indices (dAstD, F_L1 and M_A5), with models of the type:

Response variable ~ hydrological factor + biotic factor + hydrological factor : biotic factor

Were "response variable" refers to y-intercept of NAS (food web capacity) and slope of NAS (food web efficiency). To avoid multicollinearity, the predictors were centered before the calculation of the interaction term by subtracting their means. We applied the same model selection procedure than before.

Results

Fish community composition

The study area was clearly dominated by alien fishes, with an average of 79.7% of alien individuals and 82.2% of alien biomass abundances. The bleak (*Alburnus alburnus*) was the most abundant species in number of individuals, followed by the European eel (*Anguilla anguilla)*. Common carp (*Cyprinus carpio*) and again, the bleak, were the most abundant in terms of biomass. In regards to feeding functional group, most of the fish were omnivorous (86.4%), followed by the invertivorous (10.2%), the piscivorous (1.8%) and the detritivorous (1.5%) (Table 2).

Concerning average fish size, native individuals were on average 42.93 ± 3.77 mm bigger than the community mean, while alien fish were 15.47 ± 2.57 mm smaller. As expected, alien-piscivorous had a large mean body length, 344.97 ± 25.07 mm above average, and alien-preys had a mean size 26.16 ± 2.2 mm below average.

Effects of environmental and biotic variables on size structure

As shown on Table 3, y-intercept of NAS (food web capacity) was negatively related with hydrological indices describing the amplitude of sub-daily flow oscillations (dD, dSD, dpath, and dAstD) and their frequency (drev), hydrological variables estimating high (F_H5) and low (F_L1) flow events frequency and the skewness in daily flows (M_A5), as well as the percentage of alien-piscivorous fish. Thus, the greater the flow variability, the lower the y-intercept of NAS (food web capacity). Y-intercept was also positively related with the percentage of total alien individuals and percentage of alien-prey, indicating that these groups of organisms contribute to increase the amount of energy available in the ecosystem.

Response variable	*Predictor*	*AIC_0*	*AIC_f*	*Model*	*Estimate*	*SE*	*p_value*	*R^2*
y-intercept of NAS (food web capacity)	*dD*	*219.53*	*201.72*	*M2*	*-0.034*	*0.011*	*<0.01*	*0.52*
	dSD	*217.37*	*199.96*	*M2*	*-0.091*	*0.030*	*<0.01*	*0.53*
	dpath	*220.93*	*200.82*	*M2*	*-0.019*	*0.005*	*<0.001*	*0.54*
	drev	*217.37*	*202.24*	*M2*	*-0.074*	*0.024*	*<0.01*	*0.56*
	dAstD	*207.17*	*189.76*	*M2*	*-13.461*	*4.360*	*<0.01*	*0.52*
	F_L1	*219.25*	*202.18*	*M2*	*-0.100*	*0.022*	*<0.001*	*0.40*
	F_H5	*219.57*	*204.37*	*M2*	*-0.087*	*0.018*	*<0.001*	*0.31*
	M_A5	*204.75*	*184.90*	*M2*	*-15.056*	*3.326*	*<0.001*	*0.63*
	% alien	*205.95*	*183.58*	*M3*	*0.118*	*0.022*	*<0.001*	*0.31*
	% alien prey	*205.98*	*183.52*	*M3*	*0.113*	*0.021*	*<0.001*	*0.22*
	% alien piscivorous	*212.06*	*195.00*	*M3*	*-0.724*	*0.333*	*<0.05*	*0.05*
slope of NAS (food web efficiency)	*dD*	*55.14*	*43.57*	*M2*	*0.004*	*0.001*	*<0.01*	*0.49*
	dpath	*56.63*	*44.45*	*M4*	*0.002*	*0.001*	*<0.05*	*0.01*
	drev	*53.71*	*41.96*	*M4*	*0.006*	*0.003*	*<0.05*	*0.52*
	dAstD	*43.02*	*31.45*	*M2*	*1.672*	*0.570*	*<0.01*	*0.49*
	F_L1	*54.57*	*40.91*	*M4*	*0.011*	*0.005*	*<0.05*	*0.00*
	M_A5	*43.04*	*29.96*	*M2*	*1.289*	*0.439*	*<0.01*	*0.53*
	% alien	*45.87*	*28.13*	*M4*	*-0.014*	*0.003*	*<0.001*	*0.22*
	% alien prey	*45.95*	*28.39*	*M4*	*-0.013*	*0.003*	*<0.001*	*0.22*

Table 3 – Results of the models adjusted between single predictors and the response variables y-intercept of NAS (food web capacity) and slope of NAS (food web efficiency). Only significant models are shown. The column "Model" indicates: M2) Mixed Effects Model with river reach as random factor and an autocorrelation structure for time; M3) Generalized Least Squares Model with a corLin autocorrelation structure for time and M4) Generalized Least Squares Model with an AR-1 autocorrelation structure for time. AIC, Akaike information criteria; SE, Standard error. R^2 corresponds to a linear regression of fitted versus real values

The slope of NAS (food web efficiency) was significantly and positively related with some hydrological indices describing the amplitude (dD, dpath and dAstD), and frequency (drev) of sub-daily flow oscillations and daily flow variability (F_L1 and M_A5) (Table3). The slope (food web efficiency) was also strongly and negatively related with the percentage of total alien individuals and percentage of alien-prey (i.e. higher percentage of alien species and alien-preys lead to steeper slopes).

Interactions between hydrological and biotic factors

In the second part of the analysis, we investigated possible multiplicative effects between hydrology and biotic factors. To avoid redundant tests and given the high degree of autocorrelation among variables shown in Table 4, we selected dAstD as a descriptor of sub-daily flow, and F_L1 and M_A5 as indicators of daily regime flow and discarded dD, dSD, dpath, drev and F_H5 for being highly correlated with the first.

	dD	dSD	dpath	drev	dAstD	$F_{L}1$	$F_{H}5$	$M_{A}5$	% alien	% alien prey	% alien piscivorous
dD	-	**0**	**0**	**0**	**0**	0.709	0.718	0.002	0.54	0.667	0.284
dSD	**0.948**	-	**0**	**0**	**0**	0.994	0.551	0.009	0.88	0.996	0.26
dpath	**0.942**	**0.869**	-	**0**	**0**	0.11	0.137	**0**	0.926	0.953	0.363
drev	**0.788**	**0.747**	**0.860**	-	0	0.082	0.051	0	0.337	0.368	0.311
dAstD	**0.987**	**0.922**	**0.915**	**0.773**	-	0.715	0.683	0.001	0.281	0.37	0.377
$F_{L}1$	0.061	-0.001	0.257	0.278	0.060	-	**0**	0	0.262	0.246	0.122
$F_{H}5$	0.059	-0.097	0.239	0.310	0.067	**0.711**	-	0.005	0.069	0.077	0.076
$M_{A}5$	0.485	0.407	**0.625**	**0.628**	0.495	0.596	0.434	-	0.404	0.444	0.721
% alien	-0.100	-0.025	-0.015	-0.156	-0.175	-0.182	-0.290	-0.136	-	**0**	**0**
% alien prey	-0.070	-0.001	0.010	-0.146	-0.146	-0.188	-0.283	-0.124	**0.996**	-	**0**
% alien piscivorous	-0.174	-0.182	-0.148	0.164	-0.143	0.249	0.284	0.058	**-0.616**	**-0.652**	-

Table 4– Correlation matrix of the independent variables that showed significant relationship with size related variables in simple models. The shaded matrix contains Spearman's r values; the open matrix contains p values. In bold significantly correlated coefficients ($r>0.6$ and $p<0.05$).

As shown in Table 5, a highly significant multiplicative effect was found between dAstD and the percentage of alien-piscivorous fish when modelling their effects on the response variables derived from NAS (food web capacity and efficiency). These results mean that the level of one factor (in this case dAstD that describes hydropeaking) modifies the way in which the other factor (here, percentage of alien-piscivorous) affects the response variable. To visualize these results, we represented in Figure 4 the food web capacity and food web efficiency as a function of the percentage of alien-piscivorous grouping data in high dAstD (values above their mean) and low dAstD (values bellow their mean). The variables dAstD and percentage of alien-piscivorous interact in such a way that in situations of high dAstD (i.e. above 0.17) the relationship between the percentage of piscivorous and trophic chain capacity was strong ($p<0.01$ and $R^2=0.44$), whereas under low dAstD conditions, the linearity between these two parameters was lost. Similarly, the trophic chain efficiency presented a significant linearity with the percentage of piscivorous in high dAstD conditions ($p<0.01$ and $R^2=0.43$) but no linearity was found in periods of low dAstD. The interactions between the other hydrological variables ($F_{L}1$ and $M_{A}5$) and the rest of biotic factors (percentage of alien species and percentage of alien-prey) were no significant.

Response variable	Significant predictors	Model	AIC_0	AIC_f	Estimate	SE	p value	R^2
y-intercept of NAS (food web capacity)	% of piscivorous	M1	195.75	176.32	-1.491	0.385	<0.001	0.74
	dAstD				-22.376	4.387	<0.001	
	% of pisc.*:dAstD*				-17.052	4.619	<0.001	
slope of NAS (food web efficiency)	% of piscivorous	M1	41.68	24.16	0.146	0.047	<0.001	0.71
	dAstD				2.489	0.532	<0.001	
	% of pisc.*:dAstD*				2.184	0.560	<0.001	

Table 5 – Results of the models showing hydrological index dAstD, predatory pressure (% of piscivorous fishes) and their interaction affecting y-intercept of NAS (food web capacity) and slope of NAS (food web efficiency). AIC, Akaike information criteria; SE, Standard error; R^2 corresponds to a linear regression of fitted versus real values; M1 means Mixed Effects Model with river reach as random factor. (*) Indicates that predictors were centred to avoid autocorrelation.

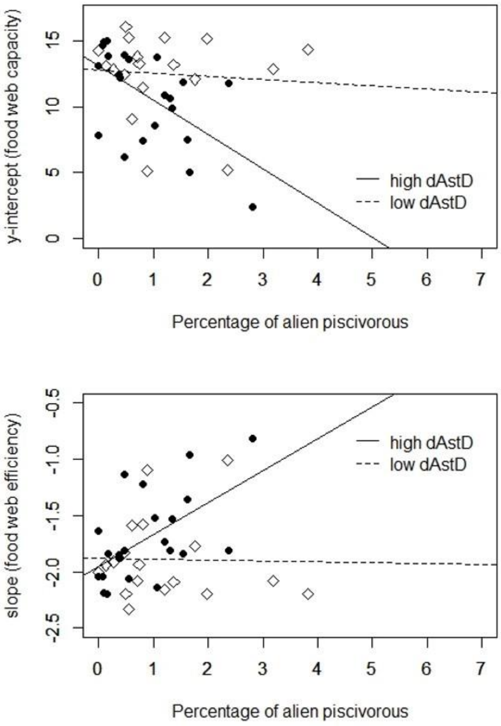

Figure 4. The response of y-intercept of NAS (food web capacity) and slope of NAS (food web efficiency) to the percentage of piscivorous is dAstD depending. Note the different grades of regression in high and low dAstD conditions.

Discussion

Our results confirm the high abundance of introduced fish species in the Ebro River previously reported by Almeida et al. (2017), Aparicio et al. (2016), Caiola et al. (2014), B. Elvira (1995a, 1995b) and López et al. (2012) among others. When we looked at how these species were represented in the size structure, we found that, although there were very large introduced species such as the Wels catfish (*Silurus glanis*) and de common carp, the most abundant alien species were characterized for being small sized fish with very high reproduction rates such as the bleak and the mosquitofish (*Gambusia holbrooki*). More concretely, 78% of small fish (lengths below the mean length of the entire community) were alien individuals while only a 22% were native fish. This high proportion of small introduced fish is related with higher values of food web capacity (i.e. higher y-intercept of NAS) and lower food web efficiency (i.e. steeper slopes of NAS). Thus, according to the models, an increase of the percentage of total alien individuals was related with an increase of food web capacity (i.e. total abundance), as well as with a decrease of food web efficiency and a greater proportion of small fish. The same happened with the percentage of alien-prey since the correlation between total alien and alien-prey was very high (r=0.996). These results indicate that total and prey alien fish contribute to increase the amount of energy available in the ecosystem.

Among the hydrological variables that we included in the analysis, only those describing variability of flow conditions were significantly related with fish community size structure. F_H5 and F_L1 are the occurrences of high and low extreme flow events while M_A5 gives an idea of the asymmetry in the distribution of flow measurements. The others are directly linked to diel flow variability, and they can be considered hydro-peaking descriptors (Bevelhimer et al., 2014) that take higher values when the amplitude of flow sub-daily oscillations increase (dD, dSD, dpath, dAstD) or the frequency of fluctuations is higher (drev). A hydroelectric power plant that operates through hydro-peaking to cover daily rises of energy demand is located 4 km upstream from the station E05. Although the hydro-peaking was not constant during the whole year, it persisted for long periods with average ranges of flow fluctuation between 165 $m^3 \cdot s^{-1}$ (in February) and 79 $m^3 \cdot s^{-1}$ (in July). The negative relationship between hydrological indices and food web capacity can be read as a decrease of total fish abundance and the portion of small fish in conditions of high hydropeaking. Other authors previously observed a decrease of small sized fishes and juveniles in the river banks immediately downstream hydroelectric power stations that operate by hydro-peaking (Bain et al., 1988; Bond and Jones, 2015; Enders et al., 2017). The causes reported to explain this decline are several: Jones (2013) found a reduced population of benthic invertebrates in the margin area influenced by dewatering, and Bond and

Jones (2015) hypothesized that this could lead to a lack of bentivorous fishes and juveniles that would move to deeper waters looking for more feeding resources. Enders et al (2017) pointed to the loss of suitable habitat, increased mortality or altered behaviour of small-bodied fishes as the causes of their observations, but they also pointed out that desiccation and scouring of spawning locations during the incubation period could suppose a big loss of individuals for the next generation. Our results further suggest that the decrease of total fish abundance and of small fish at high hydropeaking conditions may have consequences on the whole food web capacity.

Our results also showed that another biotic factor was significantly related with food web capacity and efficiency: the percentage of alien-piscivorous fish. As stated for the first time by Elton (1927) in the beginnings of trophic ecology, the predatory-prey interaction is size-depending in a manner that big fishes feed on the smaller ones. Thus, a big presence of piscivorous will reduce by consumption, the number of small sized fishes and this fact will be reflected on the shape of body-size distributions (Allen et al., 2006). Similar trends have been observed by other researchers in marine environments, (Bianchi, 2000; Rice and Gislason, 1996) as well as in lakes (Emmrich et al., 2011) and rivers (Murry and Farrell, 2014). But going deeper into the linkage of predation and size structure, our results suggest that hydro-peaking has a multiplicative effect on this relationship, so that under conditions of high flow oscillations, the predation by alien-psicivorous may become more efficient, triggering to a higher modification of size spectrum parameters (Fig. 4). Under low hydro-peaking conditions, the linearity between the amount of predators and size spectrum parameters was lost, suggesting a low efficiency of predation. This could be explained, as Boavida et al. (2013) and Bond and Jones (2015) suggested, by the action of flow fluctuation caused by hydro-peaking that would force small fishes to avoid the riverbed area that is continuously wetted and dried, moving to deeper waters where the encounters with larger piscivorous would be more frequent. Also Bain et al (1988) proposed the increase of predation risk in shallow areas during high flows as one of the most important causes of the reduction of small fish in high hydro-peaking conditions.

Conclusions

This study suggest that hydro-peaking may affect the populations of small fishes in the lower Ebro River. In addition, we observed a possible interaction between hydro-peaking and the presence of piscivorous fish. Although more investigation is required to better understand it, this interaction would make fish community size structure more susceptible to change by the action of predation in high hydropeaking conditions. These results can help water authorities and hydropower managers to consider mitigation measures for hydro-peaking impacts on fish. Furthermore, our study shows that the size structure of fish community in the lower Ebro River is highly determined by the presence of alien species.

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General discussion

This thesis focuses on the study of the effects of the hydrological regime on the fish community from the lower Ebro River. These effects were evaluated using different approaches:

- **Chapter 1** effects on single species abundances
- **Chapter 2** effects on the fish community structure
- **Chapter 3** effects on the community size spectrum.

In all three chapters, two important aspects of human action on the ecosystem of the lower Ebro River were discussed: the alteration of the natural hydrological regime and the presence of a large number of introduced species. Moreover, the relationship between these two factors, i.e. how the alteration of the hydrological regime affects the introduced species, was analysed.

The present work of monitoring fish stocks is the most complete done in the study area. It integrates data collected for more than ten years belonging to a monitoring programme carried out by the Marine and Continental Waters research group of IRTA since 2006 that continues today with an annual sampling. In addition, the data set was complemented with nine intra-annual samplings during 2014 and 2015.

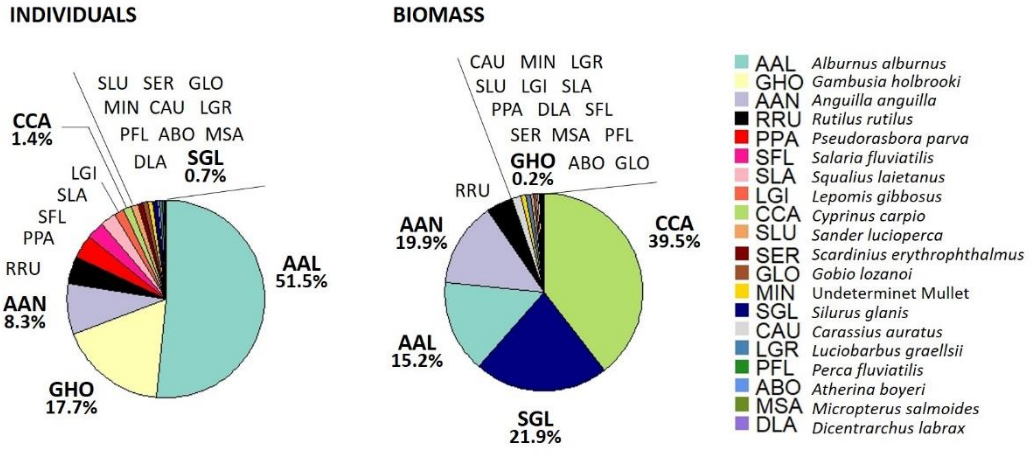

Fig. 1 Percentages of species abundances averaged for all samplings expressed as individuals and biomasses.

As can be seen in Figure 1 and has been discussed in **Chapter 1**, the fish community of the lower Ebro River is dominated by alien species, both in number of individuals and in biomass. The most abundant species were the bleak (*Alburnus alburnus*) and the mosquitofish (*Gambusia holbrooki*) in terms of individuals and the common carp (*Cyprinus carpio*) and the Wels catfish (*Silurus glanis*) in terms of biomass. Native species that supposedly should dominate the original community such as the Ebro barbel (*Luciobarbus graellsii*), the Catalan chub (*Squalius laietanus*) and the European eel (*Anguilla anguilla*) today are reduced to less than 10% of the total fish, and others like the Ebro nase (*Parachondrostoma miegii*) were not even found in this study. These results should ring serious alarm bells about conservation of native fishes. All the ichthyologists who have addressed the issue of introduced species in the Iberian Peninsula agree on the urgency of ecological restoration measures to stop the regression of endemic species that have an incalculable ecological value (Aparicio et al., 2016; E García-Berthou et al., 2015; Ilhéu et al., 2014; Leunda, 2010; Ribeiro and Leunda, 2012).

The analysis of flow-species abundances interactions carried out in **Chapter 1** brought out some interesting results that could lead to the development of flow-based tools for environmental restoration. Nine alien species and two native species were found to be significantly related with hydrological indexes for both, intra and inter-annual variations. Behind these results, specific and complex mechanisms of relationship with the environment are hidden, but as a general rule, they suggest that some invasive species such as the mosquitofish, the zander, the European perch and the topmouth gudgeon are not well adapted to high flood events as stated by other authors (Alexandre et al., 2013; Bernardo et al., 2003; Ribeiro et al., 2013). This would allow us to use the flood events as a control tool for these species and lead us to propose the increase of F_H3 (average of daily flows above 3 times the median daily flow), the decrease of M_H17 (mean of the 25th percentile from the flow duration curve divided by median daily flow) and the increase of M_A13 (average November flow) to be studied more in depth for this purpose.

Having proved the existence of significant relationships between some hydrological indices and fish species abundances, we were interested in explore how fish-based bioindicators perform with these same and other hydrological variables. With this scope, in **Chapter 2** ecohydrological relationships were analysed using three indices of biological quality assessment based on fish community: the Indices of Biotic Integrity in Catalan Rivers ($IBICAT_{2010}$; Sostoa et al., 2010 and IBICAT2b; García-Berthou et al., 2015) and the European Fish Index (EFI+; Fame Consortium, 2001). Among them, $IBICAT_{2010}$ was the one that presented more significant correlations with hydrological indexes. It was significant and negatively related with the mean of annual minimum flows (M_L14), the mean of percentile 25th divided by median of daily flows (M_L23), the standardized maximum hourly ramping rate (dstMHramp) as well as the variable depth. Also the spatial scale turned out to be important since some sampling transects showed clearer relationships than others suggesting an influence of the river hydromorphologic variability on the ecological quality scores as shown in other studies (Brunke, 2008; Elosegi et al., 2010). Moreover, temporal patterns were identified so that daily hydrological indices showed significant relationships with ecological quality when computed using between 9 and 36 months of flow records (previous to the sampling date) whereas sub-daily indices responded better when calculated with 3-9 months of records. These are shorter response times that those previously reported after common restoration measures (Roni et al., 2002).

But the effects of environmental factors can be reflected not only on the community composition but also on its size structure. In **Chapter 3** we looked for relationships between the linear parameters y-intercept and slope of Normalized Abundance Spectra (NAS) of the entire fish community and hydrological indexes during an annual cycle. In this case we included daily and sub-daily indexes because we were interested on analyzing whether or not there was any detectable effect of hydropeaking fluctuations on size structure as already suggested by other authors (Enders et al., 2017; Young et al., 2011). We found significant relationships between NAS-related parameters and the hydrological variables describing diel flow oscillations and daily flow variability suggesting that high flow variability conditions and, above all, high hydro-peaking conditions, caused a diminution of the total abundance of fish and a decrease of the proportion of small sized fish. Furthermore, a significant interaction between descriptors of hydro-peaking and the percentage of alien-predators suggested that high hydro-peaking conditions benefited predation by facilitating predator-prey encounters in accordance with previous observations (Bain et al., 1988; Boavida et al., 2013; Bond and Jones, 2015). Another interesting fact described in **Chapter 3** was that alien fish contribute to increase the amount of energy available in the ecosystem (providing higher values of food web capacity, i.e. higher y-intercept of NAS and lower food web efficiency, i.e. steeper slopes of NAS) since the most abundant alien species were characterized for being small sized fish with very high reproduction rates such as the bleak and the mosquitofish. We concluded that the high proportion of alien fishes and the presence of a hydropower generation plant that operates by hydro-peaking are important factors determining fish size structure in the lower Ebro River.

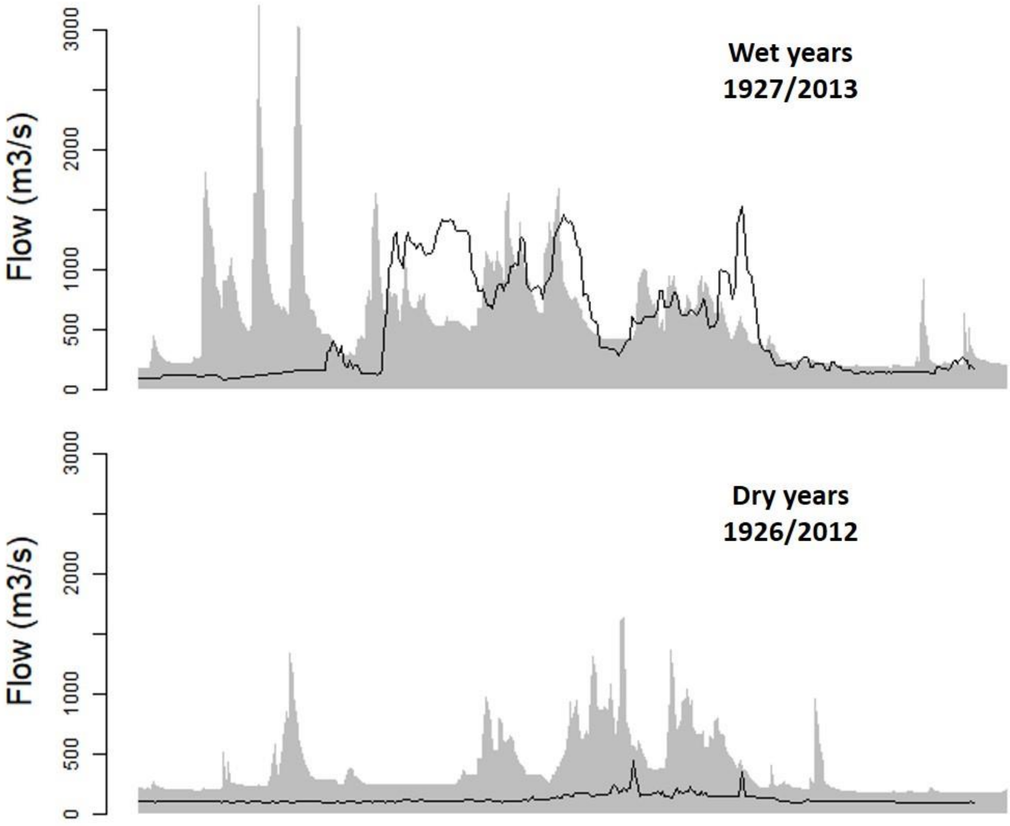

Fig. 2 Water discharge in the lower Ebro River (Tortosa) under historical (grey) and present conditions (black)

But it makes no sense to talk about the current hydrological regime without considering which its degree of alteration is with respect to the natural flow. We compared flow series before and after dam construction in **Chapter 1** and we found significant differences in several hydrological indexes. Some indexes describing rate of change between increasing and decreasing flows, low flow conditions and droughts, increased their values with dam construction (R_A8, D_L13, M_L14, M_A5 and M_H17; see definitions in **Chapters 1, 2 and 3**), while others describing high flow conditions and flood events, decreased (F_H3, M_A12, M_H20, M_A13, M_H2 and M_H1; see definitions in **Chapters 1, 2 and 3**). This is what can be expected from water management aimed at agricultural and urban supply, with storage of winter flooding and increased baseflows in summer for irrigation (Batalla et al., 2004; García and Moreno, 2000). Moreover, the set of sub-daily indexes used in **Chapters 2 and 3**, although they could not be compared with any historical series due to a lack of hourly data, it is obvious that they also underwent changes with dam construction, since there is no source of sub-daily flow variability in nature comparable in magnitude and frequency to that produced by hydro-peaking

(Bevelhimer et al., 2014; Zimmerman et al., 2010). As we have seen before, we can associate some of these indices with changes in the fish community such as abundances of mosquitofish, zander, razbora, European perch, roach, common carp, bleak, Iberian gudgeon, goldfish, Ebro barbel and pumpkinseed (**Chapter 1**), scores of the fish based bioindicator Ibicat$_{2010}$ (**Chapter 2**) and y-intercept (food web capacity) and slope (food web efficiency) of Normalized Abundance Spectra (**Chapter 3**). These results are further evidence that the hydrological alteration of dams modifies the downstream ecosystems, in some cases degrading their biological quality (**Chapter 2**; Clavero et al., 2004; Ferreira et al., 2007) and causing adverse effects on native species through favoring alien fish populations (**Chapter 1**; Merciai et al., 2017; Oliveira et al., 2018), or enhancing predation (**Chapter 3**; Boavida et al., 2013; Bond and Jones, 2015).

Nowadays, global change is perhaps the greatest challenge for science and society, as it is for freshwater ecosystems. In Europe is expected an increase in air temperature greater than the global average for the next century, together with a rise in high-temperature extremes, worse droughts and heavier torrential rains. Precipitations in Southern Europe will diminish triggering a decrease in river flows, accentuated by the higher evapotranspiration (Alba-Tercedor et al., 2017; Kovats et al., 2014). The Mediterranean Basin is one of the regions where predictions for global change prognosticate large effects on ecological quality of water bodies (Herrero et al., 2018; Navarro-Ortega et al., 2012). In addition, the imperative need to reduce the consumption of fossil fuels will probably lead to a necessary increase in the number and activity of hydroelectric plants, and therefore, of the fluctuations caused by hydropeaking (Schmutz et al., 2015). In this context of changes, the need to understand flow-ecology interactions and to protect native fish species becomes an urgent issue. Here, some research items have been proposed in this direction based on reasonably long time series of fish monitoring. But more research is needed, and we firmly believe that *in situ* experiments through the application of controlled flows accompanied by exhaustive monitoring programs would provide valuable information to design effective restoration measures for the Ebro that could be applied in other river basins. The use of designed restorative flows have been already applied in other cases providing very interesting results. For instance, in the lower Ebro, flow pulses are made regularly since 2008 as a control measure for the proliferation of macrophytes (Batalla et al., 2008; Ibáñez et al., 2012b) and in the Rhône River (France), it was decided to increase minimum daily flows to improve river connectivity (Lamouroux and Olivier, 2015). Therefore, a more intimate collaboration between freshwater scientists, management agents and policy makers is needed (Navarro-Ortega et al., 2012; Stoffels et al., 2018), as well as greater efforts in hydrological modeling of future scenarios (Radinger et al., 2018).

In this dissertation we have focused on flow as a main driver of fish community structure, but it is difficult to separate the effects of hydrological regime from those of other variables since there are many factors affecting aquatic organisms and very high complex interactions occur between them. We can't forget, for example, that several studies have found high levels of organochlorine contaminants in fish (Calvo, 2005; Huertas et al., 2016) and macroinvertebrates (Cid et al., 2010) in the area coming from a chemical industry located in Flix Reservoir. The presence of these substances has been related to alterations in the reproductive capacity, condition and vulnerability to ectoparasites of some fish species (Benejam et al., 2010; Lavado et al., 2004). The harmful effects of these pollutants are linked with flow, since large avenues can cause their release from their point of confinement to downstream waters (Bosch, 2009; Chapman et al., 2003). In recent years, a decontamination project for the area has been carried out, but there are still no conclusive evaluations of the final results of this restoration at an ecological level. Also the thermal pollution described by Prats et al. (2012, 2010) interacts with flow, becoming more evident in low water volume conditions, and could influence the distribution and growth rates of some fishes, as reported in other rivers (Ginot, 1996; Wolter, 2007). Nutrient dynamics (Torrecilla et al., 2005), macrophyte proliferation (Ibáñez et al., 2012a, 2012b), and many other biotic and abiotic variables should be taken into account when defining flow strategies for fish conservation since they all interact closely with hydrological regime.

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Conclusions

1. The Ebro River was dominated by alien fish, mainly, introduced cyprinids. The most abundant species were the bleak (*Alburnus alburnus*) in number of individuals and the common carp (*Cyprinus carpio*) in terms of biomass.
2. Several species abundances were significant and negatively related with hydrological descriptors of high flow events, indicating that alien fish species such as the Eastern mosquitofish (*Gambusia holbrooki*), the pike-perch (*Sander lucioperca*), the European perch (*Perca fluviatilis*) and the topmouth gudgeon (*Pseudorasbora parva*) are harmed by high flood events.
3. Several species of cyprinids were significantly related with October and November flows, suggesting that the first stages of their progeny are closely influenced by autumn floods.
4. Hydrological indices presented significant differences before and after dam construction. Those that increased with dam regulation were (by order of magnitude of the difference): R_A8, D_L13, M_L14, M_A5 and M_H17; and those that decreased (by order of magnitude of the difference) were: F_H3, M_A12, M_H20, M_A13, M_H2 and M_H1. Therefore, these indices can be considered indicators of hydrological alteration for the study area.
5. The hydrological indices F_H3 (average of daily flows above 3 times the median daily flow), M_H17 (mean of the 25th percentile from the hydrograph divided by median daily flow) and M_A13 (average November flow) are proposed as descriptors of hydrological alteration and should be monitored in order to assist on flow management for the control of alien species and native fish conservation.
6. IBICAT$_{2010}$ was the most effective index to find relationships between fish community and flow regime in the lower Ebro River when compared with IBICAT2b and EFI+.
7. Relationships between ecological quality assessment indices and hydrological variables were spatial dependent, suggesting the influence of other factors such as the complexity of surrounding habitat (riparian vegetation) and river geomorphology, among others.
8. The temporal scale of the study affected the detectability of ecohydrological relationships. Using 9-36 months of flow data series before the sampling,

relationships with daily indexes were found, while subdaily indices gave significant results in shorter periods (3-6 months before the sampling).

9. High hydro-peaking conditions caused a diminution of the total abundance of fish and a decrease of the proportion of small sized fish (i.e. lower y-intercept and flatter slopes of Normalized Abundance Spectra, respectively).
10. There was a significant interaction between hydro-peaking and the percentage of alien-predators suggesting that high hydro-peaking conditions benefit predation by facilitating predator-prey encounters.
11. Normalized Abundance Spectra of entire fish community showed to be a good tool for ecological status assessments since reflected hydro-peaking effects and the high presence of alien species.