Master Thesis
TVVR 21/5018

INVESTIGATING ECO-HYDROLOGICAL RELATIONSHIPS BETWEEN FISHES AND FLOW REGIMES IN SPANISH RIVERS

Celia Ramos Sánchez

Division of Water Resources Engineering
Department of Building and Environmental Technology
Lund University

Investigating eco-hydrological relationships between fishes and flow regimes in Spanish rivers

By:
Celia Ramos Sánchez

Master Thesis
Division of Water Resources Engineering
Department of Building & Environmental Technology
Lund University
Box 118
221 00 Lund, Sweden

Water Resources Engineering
TVVR-21/5018
ISSN 1101-9824

Lund 2021
www.tvrl.lth.se

Master Thesis
Division of Water Resources Engineering
Department of Building & Environmental Technology
Lund University

Swedish title: Undersöker ekohydrologiska samband mellan fiskar och flödesregimer i spanska floder.

English title: Investigating eco-hydrological relationships between Fishes and flow regimes in Spanish rivers

Author(s): Celia Ramos Sánchez

Supervisor: Erik Nilsson

Supervisor: Magnus Larson

Examiner: Rolf Larsson

Language English

Year: 2021

Keywords: Eco-hydrological relationships, Fish metrics, Hydrological metrics, Environmental flows, Hydrological alteration

Acknowledgements

I collected and analyzed the data presented in this Master's thesis during an internship at Tragsatec Company, in Madrid, Spain. I want to thank Fernando Magdaleno and Alfredo Corrochano for the internship opportunity. I would also thank The Ministry for the Ecological Transition and the Demographic Challenge, including Belén Calleja, for providing the hydrological data, and Tragsatec company, for letting me take part in the field campaign where the fish data was collected.

My special thanks go to all the members of the team e-flow, including Gabriel Mezger and Joaquín Soler, who welcomed me, helped me, and made the field campaign so special and memorable. I learned a lot from each of you.

I want to thank my supervisors, Magnus Larson, and Erik Nilsson at the Division of Water Resources Engineering, for being very comprehensive and flexible with my time constraints and last-minute changes, for their detailed corrections, and transmitted knowledge. It was highly appreciated and helpful during my project work.

Lastly, I want to express my thanks and gratitude to my supervisor at Tragsatec, Juan Diego Alcaraz, for introducing me to this research topic, transmitting to me his passion for rivers, and always being a very supportive and knowledgeable supervisor throughout the different stages of my degree project. Thanks for your willingness to help me always and your advice.

Popular summary

Regardless their benefits for the society, dams substantially alter the streamflow in rivers. The implementation of environmental flows is a management tool to reduce this alteration. There is currently a need in Spain for monitoring the effects of the environmental flows implemented in rivers. Eco-hydrological relationships provide information about how biological elements are influenced by the streamflow, and they are relevant knowledge to design environmental flows strategies. Fishes are the biological element more sensitive to flow regime changes, and thus the study of fish-flow relationships are particularly relevant in the design of environmental flows. This study presents an analysis of the relationships between flow regimes in 33 Spanish rivers, defined using a set of annual, monthly, seasonal, and daily hydrological metrics, and fish assemblages, characterized through metrics that describe features in rivers such as the number of species, the density of fishes or their habitat preference. A selection of fish and flow metrics that were statistically relevant and non redundant was conducted through a statistical analysis. Fish-flow relationships were discussed and stablished based on findings in the literature. The results showed that flow magnitude in April was related to rivers with higher number of species. Fluctuations in flow magnitude tended to decrease fish density in rivers, while rivers with variability of high flows tended to have higher values of fish density. Fish metrics related to ecological features showed some relationships with hydrological metrics in the statistical analysis, but they were not reliable enough, as the findings were not supported by the literature.

Abstract

There is currently a need in Spain for monitoring the effects of the environmental flows. The use of eco-hydrological relationships to design both environmental flows regimes and the monitoring programs that track their effects on riverine ecosystems is highly recommended. Fishes are the biological element more sensitive to flow regime changes, thus the relationship between fishes and flow dynamics becomes especially relevant to design environmental flows and the required monitoring programs. This study presents an analysis of the relationships between flow regimes in 33 Spanish rivers, defined using a set of annual, monthly, seasonal, and daily hydrological metrics, including the Indicators of Hydrological Alteration, and fish assemblages, characterized through a combination of taxonomic community composition and ecological traits metrics. A selection of fish and flow metrics that were statistically relevant and non-redundant was conducted, through a Principal Component Analysis and correlation analysis. The potential biological implications of the fish-flow relationships that were found in the statistical analysis were discussed according to the findings in the literature. The results showed that larger rivers tended to have a greater fish richness and a lower fish abundance than smaller rivers. More specifically, rivers that have their annual maximum flows in April were related to a higher fish richness, showing the importance of the timing, and not only the magnitude, of the flow regime. Furthermore, this flow condition may enhance native species richness and control the spread of alien species in rivers downstream from dams. The number of flow reversals was especially suitable when studying fish-hydrological relationships at a national scale, and they were negatively correlated with fish abundance in rivers. In contrast, flow skewness was positively correlated with fish abundance in rivers, and they may promote fish recruitment. Other correlations among some hydrological metrics and fish metrics related to ecological traits were observed and discussed too.

Table of Contents

1. Introduction

1.1. Background

In Mediterranean regions, the need for satisfying water demands that are out-of-phase with the natural seasonality of rainfall-runoff has led to rivers being more impounded than those in humid regions (Hooke, 2006; Kondolf & Batalla, 2005; Grantham et al., 2013). Despite their socio-economic benefits, dams induce multiple pressures in regulated rivers and their associated ecosystems that affect adversely their ecological integrity. The ecological integrity refers to the ability of an ecosystem to support and maintain a community of organisms that has species composition, diversity and functional organization comparable to those of natural habitats (Parrish et al., 2003). Some significant pressures caused by dams are the natural flow regime alteration, the stream water quality alteration (World Commission on Dams, 2000) and the sediment dynamics alteration (Kondolf, 1997). All these pressures are interrelated and occur in concert. However, the natural flow regime alteration has been recognized as the most critical pressure (Bunn & Arthington, 2002), as the flow regime influences directly the ecological integrity of rivers, and indirectly, through other ecological regulators (Poff et al., 1997) (Figure 1).

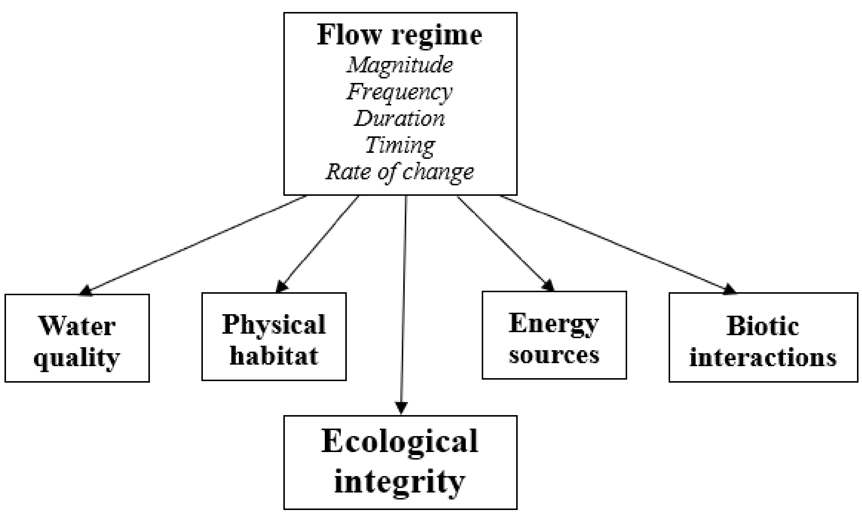


Figure 1. Flow regime directly influences the ecological integrity of riverine ecosystems and indirectly, through other elements regulators (Poff et al., 1997).

Spain, with over 1200 large dams (MITECO, 2021b) and a total capacity of 55,622 hm^3 (MITECO, 2021b), has the fifth highest number of dams in the world (WCD, 2000). More than 1000 dams were built during the second half of the 20th century (MITECO, 2021a), meaning that many rivers in Spain have now experienced hydrological regulation for more than 20 years. Studies assessing the hydrological alteration of Spanish rivers indicate significant changes in magnitude and timing of flows after dam construction (Mezger et al., 2021). In general terms, the hydrological alteration occurring in Spanish rivers due to impoundments is characterized by: (i) in drier basins, a sharp decrease in mean annual flows and extreme annual flows; (ii) in wetter basins, no common changes in mean annual flows, a decrease in the maximum daily flow and the 95th percentile flow, and a significant increase in the minimum daily flow and the 5th percentile flow (Mezger et al., 2021). In most cases, the natural seasonality of the annual hydrographs in the Mediterranean is inverted, with the high-flow period occurring during the low precipitation months (summer) and the low-flow period during the wet season (Mezger et al., 2021). The inversion of the hydrographs is usually due to the agricultural water allocations during the drier months. In Spain, hydro morphologic alterations and water diversions represent 49% of the total significant pressures hindering the achievement of good ecological state of rivers, according to the River Basin Management Plans of the second planning cycle (2015–2021). The ecological effects of hydrological alteration have been extensively documented in Spain (e.g. García de Jalón et al., 1992).

From a regulatory perspective, there is the key objective of achieving “a good ecological status” in all European water bodies as established by the Water Framework Directive (2000/60/EC). In addition, specific purposes of the Water Framework Directive require member states to prevent further deterioration of water resources and enhance their ecological status through specific measures (WFD, 2003). A widely accepted key measure to mitigate hydrological alteration is implementing environmental flows (e-flows) (B. D. Richter et al., 2006; Acreman & Ferguson, 2010). A definition of e-flow commonly used nowadays is “the quantity, timing and quality of water flows required to sustain freshwater and estuarine ecosystems and the human livelihoods and well-being that depend on these ecosystems” (Brisbane Declaration, 2007). Following this definition, the practical design and implementation of e-flow regimes in rivers should include all the elements of the natural flow regime that play a role in the ecological integrity of rivers, namely magnitude, timing, duration, frequency, and rate of change, (Acreman et al., 2014; Junk et al., 1989; Poff et al., 1997; Richter et al., 1996). For instance, e-flow regimes should include flows similar in magnitude and time (moment in the year) to overbank flows, which are essential flow elements that maintain habitat heterogeneity in space and time in riverine ecosystems (Ward, 1998).

In Spain, e-flows were first legally required in 2001, according to the River Basin Management Plans (2001). In 2008, e-flows regimes were redefined according to the Water Framework Directive, which regulated a progressive implementation throughout three 6-year planning cycles (2009–2014; 2015–2021; 2022–2027). The actual implementation of the e-flows started in 2013, and so far, they have had limited effect in alleviating the hydrological alteration. Studies examining the streamflow regimes before and after e-flows implementation show almost no variation (Mezger et al., 2021). The following two ecological aspects in the design and monitoring of e-flows have been identified as limiting factors.

Firstly, the design of e-flows did not conduct specific analyses of the causal links between pressure (i.e. natural flow regime alteration) and ecological status of the rivers, nor was the ecological response of the implemented regimes monitored and evaluated (Mezger et al., 2019). Studying and quantifying eco-hydrological relationships between streamflow regimes and ecological elements is crucial to design e-flow regimes and the indicators that allow for monitoring and assessment their effects on the ecological health of rivers (Poff et al., 2010). This is a challenge (Poff et al., 2003; Arthington et al., 2006), which also is not limited to the Spanish case (Ramos et al., 2018).

Secondly, the initial assessment of the ecological status of the rivers in Spain that set the baseline for the definition of e-flow regimes did not use fish-related indicators in any of the river basin districts, except in the Jucar Basin District (DGA & CEDEX, 2018). This is a shortcoming since fishes have been recognized as the biological element most sensitive and responsive to flow regime alteration (e.g., Poff & Zimmerman, 2010). For instance, impoundments disturb the reproductive strategies and cycles of native species (Freeman et al., 2001; Lytle & Poff, 2004), because they affect longitudinal streamflow connectivity. Consequently, declines in abundance and geographical distributions occur (Aparicio et al., 2000; Clavero et al., 2004). This sensitiveness and response at wide temporal and spatial scales make fishes common bioindicators for assessing the ecological health of rivers (Colin et al., 2016; Hermoso et al., 2009). For this reason, fishes were used to develop the first method to estimate the biotic integrity of riverine ecosystems (e.g., Karr, 1981; Fausch et al., 1984). Hence, especially in regulated rivers, it is crucial that eco-hydrological relationships are studied primarily for fish assemblage (Balcombe et al., 2011).

Consequently, there is currently a need in Spain for tracking the ecological effects of the implemented e-flows on rivers and associated ecosystems to contribute to improved e-flows regimes in the third and last planning cycle of the Water Framework Directive (2022–2027). This study is framed within the project “Monitoring the effect of the environmental flow regimes established by the Spanish River Basin Management Plans on the intercommunity rivers”, which is addressing this need, and it is conducted at Tragsatec Company for The Ministry for the Ecological Transition and the Demographic Challenge. One of the parts of this project studies eco-hydrological relationships between biological indicators (e.g. fish, macroinvertebrates, macrophytes, etc.) and hydrological regimes, as it is essential knowledge to design appropriated e-flows.

1.2. Aim

The purpose of this study is to investigate eco-hydrological relationships between the biological fish community and hydrological flow regimes in Spanish rivers. The focus lies on fishes because it is the biological indicator most sensitive to hydrological alteration. Quantitative eco-hydrological relationships are commonly studied by statistically analyzing the relationships between biological and hydrological metrics. Therefore, the two specific objectives of this study are: (i) to identify hydrological and biological metrics to characterize the e-flow regimes and the fish information, respectively; (ii) to assess the relationships between the selected metrics to investigate links between fishes and flows.

Outcomes from this study will set the basis for the study of fish-hydrological relationship part of the project at Tragsatec company, and contribute to its overall objective.

2. Material and methods

2.1. Study area

The study was conducted in 33 rivers in Spain distributed over ten river basin districts (Figure 2). All river basins districts are under state administration because they are part of rivers that extend to more than one Autonomous Community. Together they cover an area of 435,572 km^2, 86% of the total Spanish area. The available surface water resources in the basin districts are 79,691 hm^3/year on average, simulated by the conceptual and quasi-distributed SIMPA model (Álvarez et al. 2004; Estrela and Quintas 1996) for the period (1980/81-2006/07), which corresponds for 73% of the total surface water resources of Spain. The total water demand is approximately 26,644 hm^3/year, or 87% of the total demand in Spain, with agriculture (85%), domestic (12%) and industry (3%) as the primary water uses. The water stress in the river basin districts is varied. The Water Exploitation Index plus (WEI+), which reflects the total freshwater use as a percentage of the renewable freshwater resources (groundwater and surface water), illustrates the notable hydrological heterogeneity of the studied area (Figure 2). Values above 20% reflect water stress, while values above 40% indicate severe stress and long-term unsustainable resource use (Raskin et al., 1997). Thus, four out of the ten river basins district are under severe hydric stress and long-term unsustainable use of the water resources.

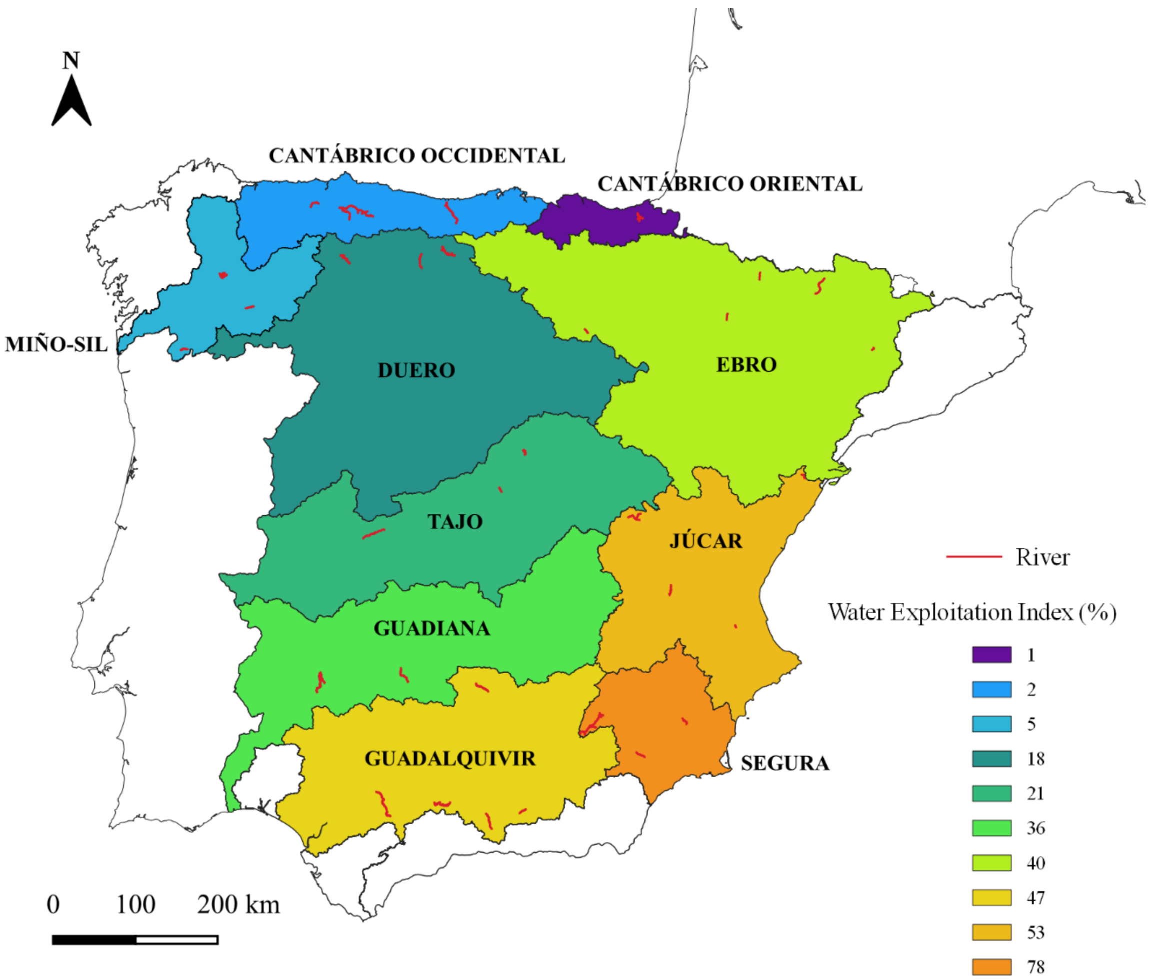


Figure 2. Distribution of the 33 rivers and location of the river basin districts. Colors show the values of the Water Exploitation Index for each river basin district.

The specific studied rivers were selected according to the following criteria:

- Representative of a national gradient of hydromorphological, ecological and pressure conditions in regulated bodies. This means that, within the Spanish territory, the selected rivers are as different as possible among themselves in terms of hydromorphology, ecological conditions and sort of pressures. This ensured to have a river sample as heterogeneous as possible, even though the these features were not relevant for this study.
- Located downstream from dams that have e-flow regimes approved in the River Basin Management Plans of the second planning cycle (2015–2021).

- The river flow should not be larger than 5% more than the respective dam discharge; this is to say that the river flow was not influenced by any important tributary, discharge, or water diversion.
- Priority was given to rivers with protected areas or species that may be significantly affected by e-flow regimes
- Priority was given to rivers that have historical ecological monitoring that will provide required information for the project where this study is framed but not for this specific study.

The author of this study did not carry out the selection process. It was carried out by another member of the team, throughout a methodology that included rivers of interest for the River Basin Authorities.

2.2. General method

Eco-hydrological relationships are normally investigated by statistically analyzing relationships between pair of biological and hydrological metrics that quantify aspects of a biological community and a flow regime respectively. Metrics are quantitative measures that represent a characteristic of either a biological indicator, in this case, the fish community, or a streamflow regime. They are use to build Indices, which combine several sorts of metrics to provide a more comprehensive and holistic view of a biological community (EPA, 2011). This study only worked with metrics, as indices generally gives information about the ecological quality of an aquatic ecosystem, which is out of the scope of this study. The abundance of both biological and hydrological metrics available for evaluation makes it challenging to select the appropriate metrics that have biological relevance, and unique and non redundant patterns of variance (Olden & Poff, 2003). The approach followed in this study combined the selection of relevant fish and hydrological metrics from the literature, followed by a final selection of metrics based on their statistical properties. Firstly, a set of biological and hydrological metrics were proposed based on the literature. Secondly, the number of biological and hydrological metrics was reduced, excluding highly correlated metrics and metrics that showed no fish-flow relationship. Lastly, fish-flow relationship among the selected metrics were analyzed. Metrics are computed in the R programming language (R Core Team, 2019).

2.3. *Hydrological data*

For the 33 selected rivers, 33 gauging sites, either station or dam, were used. Daily series were obtained by request to the River Basin Authorities and the hydraulic intracommunity administrations through the Center for Hydrographic Studies from the Centre for Public Works and Experimentation (CEDEX). Ideally, the hydrological metrics should be calculated for a period of 20 years as recent as possible (1999-2020), to represent the current hydrological variability of a stream flow, including extreme events such as floods and droughts (Worrall et al., 2014; Taylor et al., Unpublished; B. Richter et al., 1997). However, this was not possible due to two main reasons. First, some of the series contained years with more than 30 consecutive days of unavailable data, which are not recommended to get reliable results (Poff et al., 2010). Second, some of the dams were built after 1999. Given these limitations, a selection of appropriate years for every station was performed in R, using the function *fillMiss* from the package *waterData* (Karen & Aldo, 2017). This function fills in the years where missing data is less than 40% and spread through less than 30 consecutive days by using a structural time series function. During this phase, the author received substantial help from another member of the team. Table 1 shows the selected study periods for each river.

Table 1. Selected hydrological period for the studied rivers.

River	Period	Number of years
Urumea II	2010-2020	10
Nansa III	2010-2020	10
Nalon III	1998-2017	19
Narcea V	1998-2017	19
Xares III	2012-2020	8
Mao II	2012-2020	8
Salas II	2005-2020	15
Riaza	1998-2018	20
Carrion - Compuerto Dam	2013-2018	5
Pisuerga	2013-2018	5

Luna	2013-2018	5
Carrion - Velilla Dam	1998-2018	20
Tietar	1998-2018	20
Lozoya	1998-2018	20
Manzanares	1998-2018	20
Matachel II	2009-2017	8
Guadamatilla II	2009-2018	9
Montoro	1998-2018	20
Cacin	2000-2018	20
Genil	1998-2018	20
Aguas Blancas	1998-2018	20
Corbones	1998-2018	20
Luchena	1998-2018	20
Segura	1998-2018	20
Jucar - La Toba Dam	2010-2020	10
Jucar - Tous Dam	1998-2018	20
Cenia	1998-2018	20
Cabriel	1998-2018	20
Gallego - San Julian Ravine	1998-2018	20
Lumbreras	1998-2018	20
Esera	1998-2018	20
Gallego - Bubal Dam	1998-2018	20
Segre	2010-2020	10

Once the hydrological data was processed, visual observation of the hydrographs allowed to have an overall idea of the type of hydrological regimes of each river. The hydrological regimes of the rivers varied considerably. On the one hand, some rivers showed hydrological patterns not far from the natural hydrological regime in Mediterranean areas (see example in Figure 3.b and Figure 4.a). On the other hand, some rivers presented a clearly impaired hydrological regime, with almost no variability of flow over the years (Figure 3.a), inverted hydrological regimes due to agricultural use (Figure 4.b) and high differences in their daily flows due to hydropower generation (Figure 4.c).

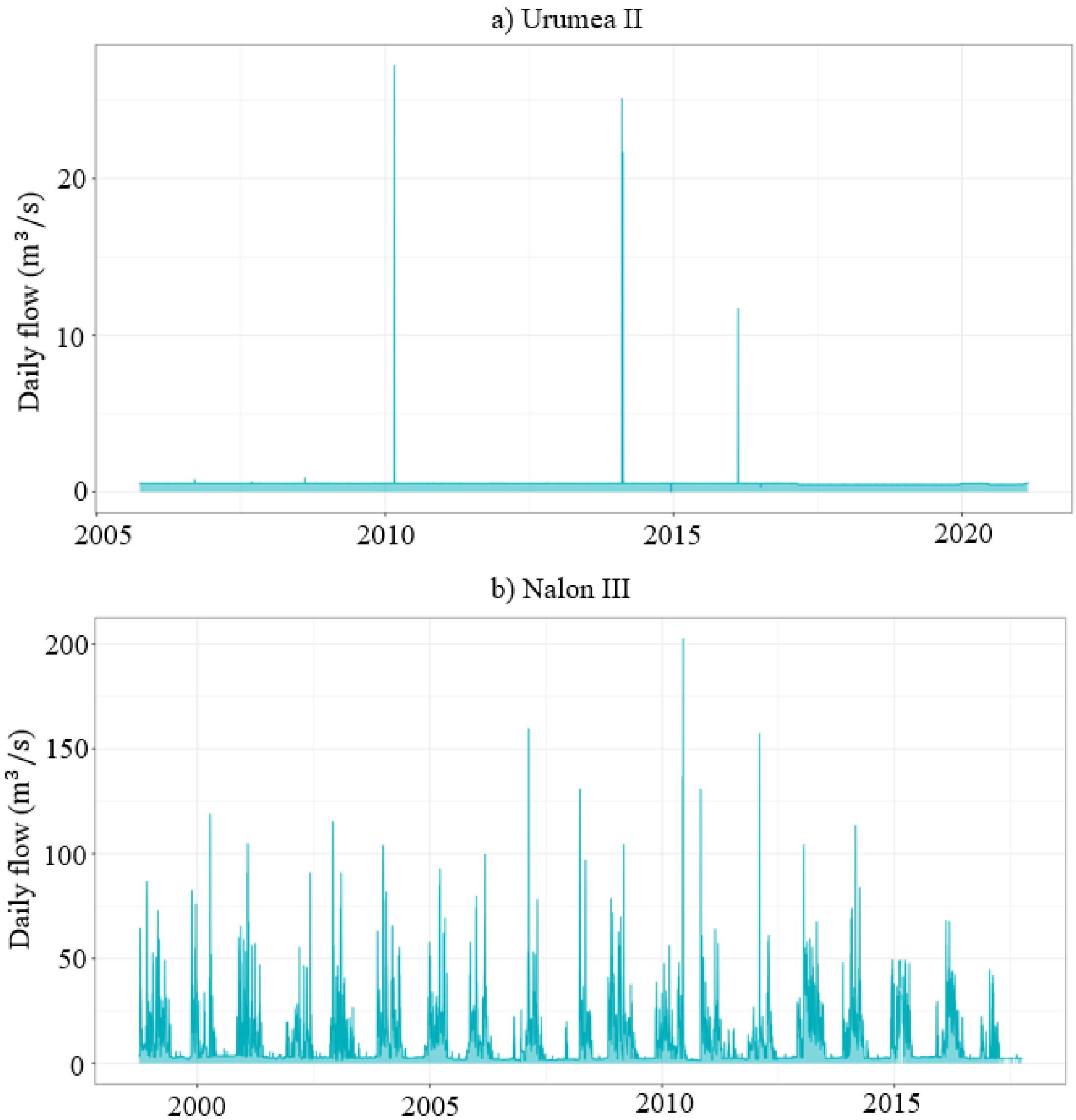


Figure 3. Two examples of hydrological datasets. Urumea II River (a) presents highly impaired flow regime with almost no variation due to hydropower generation. Nalon III River (b) presents patterns similar to the natural hydrological regime in template areas.

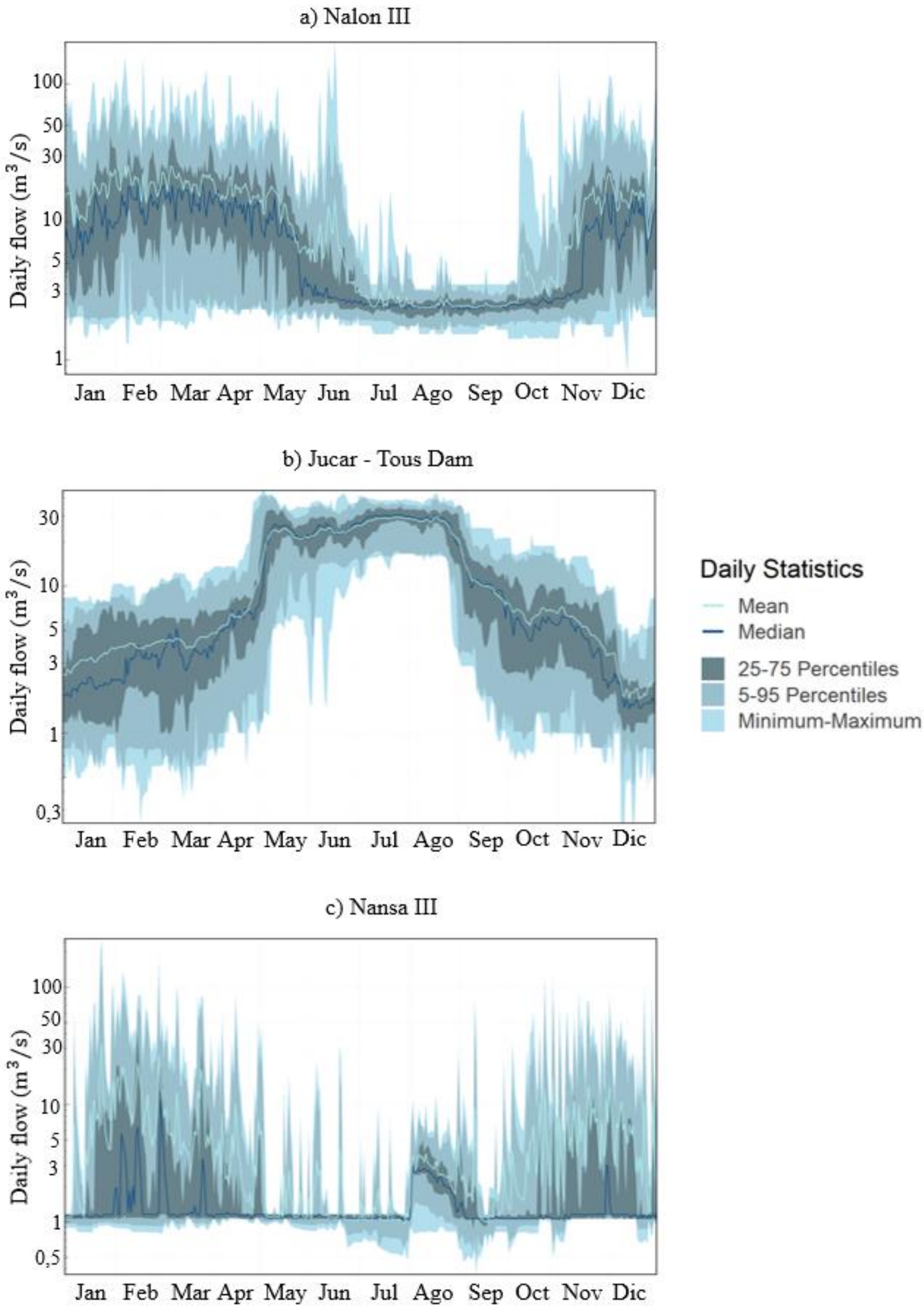


Figure 4. Three examples of annual hydrographs with daily statistics. Nalon III (a) presents patterns similar to the natural hydrological regime in template areas. Jucan -Tous Dam (b) presents an inverted hydrograph caused by agricultural water use. Nansa III (c) presents an inverted hydrograph localized in August with constant minimum flow of 1 m^3/s throughout the year and maximum flows with high fluctuations, likely caused by agricultural water use in August and hydropower water use throughout the year.

2.4. Hydrological metrics

The selection of the hydrological metrics was done through literature review. The following criteria guided the literature review to find metrics that: (i) describe the full range of variability of the hydrological regime (Mathews & Richter, 2007), (ii) are derived from ecological principles regarding influences of flow on fish communities (Arthington et al., 2006), (iii) incorporates essential aspects of the flow regime shared across particular rivers types that allow to study them in common and been validated with biological data.

A total of 40 metrics were selected. Among them, 33 were the so-called Indicators of Hydrological Alteration, a set of hydrological metrics proposed by Richter et al., (1996) to characterize the hydrological flow regime as a previous step for assessing hydrological alteration in rivers. These metrics have been used widely in the study of eco-hydrological relationships and have provided the fundaments for the development of other metrics and more complex approaches (e.g. Yarnell et al., 2020). In addition, 7 metrics were selected, based on metrics used in previous studies relevant to fish community in Mediterranean-climate rivers. The temporal scale of all the metrics is either annual, seasonal, monthly, or daily, as there were no available hydrological data series at a shorter temporal scale. The description of the selected metrics, together with the reference to the studies that used them, are listed in Table 2. Figure 5 defines graphically the flow components that are used to compute the hydrological metrics of Table 2.

The selected hydrological metrics were classified into 5 groups (Table 2), following the common classification based on the five key components of the natural flow regime that regulate ecological processes in river ecosystems: magnitude, timing, duration, frequency, and rate of change (Poff et al., 1997; Richter et al., 1996). Magnitude is simply the amount of water moving passing through a fixed river site per unit time. Frequency refers to the periodicity for a certain event to occur in specific period (e.g., the 5-year flood). Duration is the time associated with specific flow condition (e.g. number of days of zero flow). Timing is the regularity with which specific flow conditions happen (e.g. Julian day of first peak flow in autumn). Finally, the rate of change refers to the speed at which flow magnitude varies (Poff et al., 1997; Richter et al., 1996).

Group 1, magnitude of monthly and seasonal water conditions, are related to habitat availability, and it influence basic features as water temperature and oxygen levels in the water column. Group 2, magnitude and duration of annual extreme flow conditions, determines the structure of the ecosystem, including the morphology of the riverbed, the availability and accessibility to floodplains and the longitudinal and transversal connectivity. It also influences biotic factors such as vegetation density that provide refuge for small-size fishes. Group 3, timing of annual extreme flow conditions, are related to life history strategies and behavioral mechanisms, for instance, access to habitat during reproduction or to safe placed to avoid predation. Group 4, frequency and duration of high and low pulses, refers to increases or decreases regarding the dominant flow conditions, without overcoming the channel banks or breaking the longitudinal connectivity, respectively. They are important to oxygenate the river water, relief warm temperatures, deliver food for fishes such as macroinvertebrates and improve the access to up/downstream sites. Finally, Group 5, rate and frequency of flow conditions changes, are related to stability conditions of the habitat.

The Indicators of Hydrologic Alteration were computed through the Indicators of Hydrologic Alteration software, developed by The Nature Conservancy in the 1990s to quickly process daily hydrologic records (Mathews & Richter, 2007). The computation of the extra 7 metrics was performed using the package *hydrostats* (Nick, 2019) for some of them. The computation of metrics followed a non-parametric approach, as the distribution of the data were not normally distributed. Consequently, medians were used instead of means.

Table 2.Hydrological metrics computed to characterize hydrological regimes of the rivers

Acronym	Description	Reference
Group 1: Magnitude of monthly and seasonal water conditions		
Q_OCT, Q_NOV, Q_DIC, Q_JAN, Q_FEB, Q_MAR, Q_APR, Q_MAY, Q_JUN, Q_JUL, Q_AUG, Q_SEP,	Median value for each calendar month (m^3/s)	(Fornaroli et al., 2020) (Carlisle et al., 2017) (Belmar et al., 2018)
AUT_Q, SPR_Q, WIN_Q, SUM_Q	Median value for each season (m^3/s)	(Dibble et al., 2015) (Carlisle et al., 2017) (Fornaroli et al., 2020)
Group 2: Magnitude and duration of annual extreme flow conditions		
Q_1MIN, Q_3MIN, Q_7MIN, Q_30MIN, Q_90MIN, Q_1MAX, Q_3MAX, Q_7MAX, Q_30MAX, Q_90MAX	Median annual minima, 1-day, 3-day, 7-day, 30-day, 90-day (m^3/s) Median annual maxima, 1-day, 3-day, median 7-day, 30-day, 90-day (m^3/s)	(Yarnell et al., 2020) (Balcombe et al., 2011) (Belmar et al., 2018)
N_QZERO	Number of zero-flow days (days)	(B. Richter et al., 1996)
Q_BASE	Base flow index: 7-day minimum flow/mean flow for year (unitless)	(B. Richter et al., 1996)
Group 3: Timing of annual extreme flow conditions		
Q_JULIANMIN, Q_JULIANMAX	Julian date of each annual 1-day maximum and minimum day (date)	(Yarnell et al., 2020) (B. Richter et al., 1996) (Fornaroli et al., 2020)
Group 4: Frequency and duration of high and low pulses		
N_LOWPULSE, N_HIPULSE	Number of low and high pulses within each water year (units)	(B. Richter et al., 1996) (Fornaroli et al., 2020)
DUR_LOWPULSE, DUR_HIPULSE	Mean duration of low and high pulses (days)	(B. Richter et al., 1996) (Fornaroli et al., 2020)
Group 5: Rate and frequency of water condition changes		
RISE, FALL	Rise rate and fall rate: median of all positive and negative differences between consecutive daily values (unitless)	(B. Richter et al., 1996)
REVERSALS	Number of hydrologic reversals: number of time that the flow increases and decreases in consecutive days (units)	(Carlisle et al., 2017) (B. Richter et al., 1996)
SKEWNESS	The average annual maximum / mean daily flow (unitless)	(Carlisle et al., 2017) (Belmar et al., 2018)
Y_VAR	Variability across annual flows: Difference between percentiles 90 and 10 divided by median (%)	(Belmar et al., 2018)
Y_CV_p75	Coefficient of variation of the annual mean of the flow > 75 percentile (%)	(Carlisle et al., 2017)

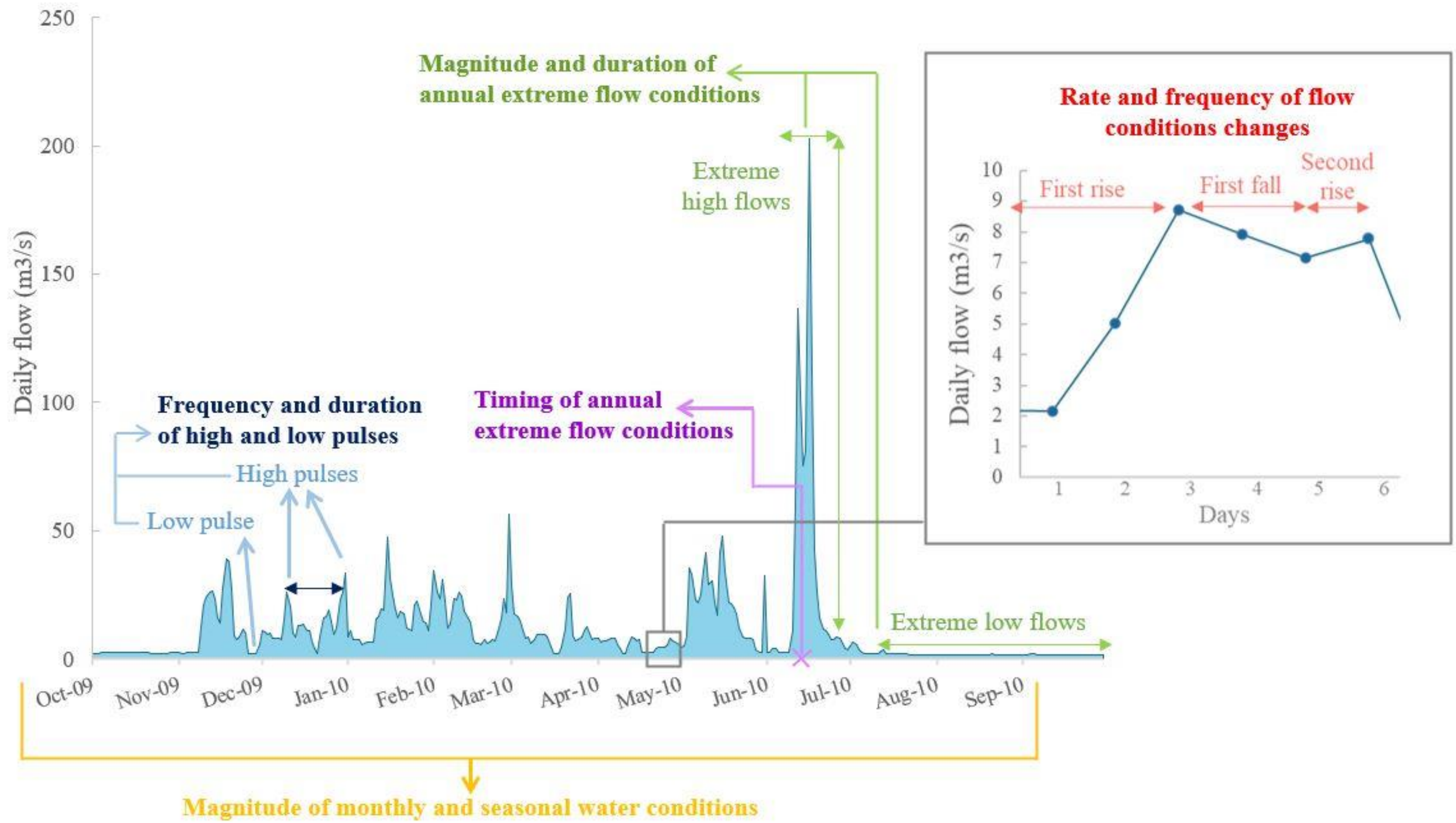


Figure 5. Graphical representation of flow components used for hydrological metrics and categorization of them by the 5 groups (yellow, green, blue, purple, and red).

2.5. Fish data

Fish sampling was conducted through electrofishing according to the protocol ML-R-FI-2015, established by the Ministry for the Ecological Transition and Demographic Growth and included in the Royal Decree 817/2015 according to the requirements of the WFD. The field campaign took place during the early spring of 2021 (March and April). This period is characterized by the beginning of the spawning season, which was evident as physical signals of reproduction were frequently observed in some individuals. The specific stretch in every river where the sampling was carried out was selected based on accessibility, security (i.e., fordable river), and representativeness. This way, fishes were sampled in accessible and safe stretches that covered the habitat variability of the corresponding river to ensure that all resident communities were included.

Electrofishing was conducted by three people, two members of team and the author of this study. One person handled the anode, and the two others caught the fishes with wooden fishnets and carried a plastic bucket for transporting the fished individuals. The voltage and the intensity were around 400 V and 1.5 A. The fishing direction was downstream to upstream, moving from one riverbank to another in zigzag to cover the entire wet width, following the single-pass electrofishing approach. Fishes were kept in plastic tanks with river water for immediate processing. Data about species level, standard length (mm), fork length (mm), total length (mm), weight (g) and record of deformities or other anomalies were gathered. Along with expert knowledge, the book from Doadrio et al., (2011) supported species identification. The author helped the fish expert to identify species. Individuals from the specie *Anguilla anguilla* were anaesthetized with clove essential oil to facilitate their measurement and release stress in the individuals. All fishes from all species were returned to the river immediately after they were measured and weighed. There was no mortality during the sampling period. In addition, habitat descriptors such as stretch width and length, percentage of shadow and number of pools and riffles, were collected, which help characterize the fluvial habitat and the computation of metrics and indices. The sampling data were complemented with pictures (Figure 6 and Figure 7).

A total of 1,743 fish individuals were collected and identified. These fishes corresponded to 7 families, 23 species of which 19 species are endemic to the Iberian Peninsula and 4 are exotic. The list of species is shown in Table 3.

Figure 6. Specimen of Salmo trutta above ictiometer to be measured.

Figure 7. Riverbed from the sampled stretch in Lozoya River. Example of picture for habitat characterization.

Table 3. List of identified species.

Orden	Family	Scientific name	Popular name
Cypriniformes	Cyprinidae	Achondrostoma arcasii	Bermejuela
Cypriniformes	Cyprinidae	Alburnus alburnus	Bleak
Anguilliformes	Anguillidae	Anguilla anguilla	European eel
Cypriniformes	Cyprinidae	Barbus haasi	Iberian redfin barbel
Cypriniformes	Cobitidae	Cobitis calderoni	Northern Iberian spined-loach
Cyprinodontiformes	Poeciliidae	Gambusia holbrooki	Mosquitofish
Cypriniformes	Cyprinidae	Gobio lozanoi	Pyrennean gudgeon
Cypriniformes	Cyprinidae	Iberochondrostoma lemmingii	Pardilla
Cypriniformes	Cyprinidae	Luciobarbus bocagei	Barbo ibérico
Cypriniformes	Cyprinidae	Luciobarbus graellsii	Ebro barbel
Cypriniformes	Cyprinidae	Luciobarbus sclateri	Andalusian barbel
Perciformes	Centrarchidae	Micropterus salmoides	Black bass
Salmoniformes	Salmonidae	Oncorhynchus mykiss	Rainbow trout
Cypriniformes	Cyprinidae	Parachondrostoma arrigonis	Loina
Cypriniformes	Cyprinidae	Parachondrostoma miegii	European nase
Cypriniformes	Cyprinidae	Phoxinus bigerri	Piscardo
Cypriniformes	Cyprinidae	Pseudochondrostoma duriense	Boga del Duero
Cypriniformes	Cyprinidae	Pseudochondrostoma polylepis	Boga del Tajo
Perciformes	Blenniidae	Salaria fluviatilis	Freshwater blenny
Salmoniformes	Salmonidae	Salmo salar	Pink salmon
Salmoniformes	Salmonidae	Salmo trutta	Brown trout
Cypriniformes	Cyprinidae	Squalius alburnoides	Iberian-Roach
Cypriniformes	Cyprinidae	Squalius pyrenaicus	Iberian-Chub

2.6. Fish metrics

The selection of fish metrics was based on a literature review. Metrics that have proved to be sensitive to hydrological and hydromophological alteration were selected. The focus lied on studies carried out primarily in the Iberian Peninsula (Spain and Portugal), although some studies from other Mediterranean, semi-arid or even central European regions were also considered relevant.

The first index for evaluation of river ecosystem quality was the Index of Biotic Integrity (IBI), developed by Karr (1981) and Karr et al., (1986) in the 1980s. It is based on the biotic integrity concept, which is “the ability to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of the natural habitat of the region" (Karr & Dudley, 1981). Based on the IBI, various fish-based indices have been developed for specific regions, being especially popular in the USA but also in several parts of Europe, including Spain. Another strategy for evaluating aquatic ecosystems health is based on comparing observed fish assemblages to the specific fish reference assemblage that would exist under undisturbed or near-undisturbed conditions, which is referred to as "the reference condition approach" (Bailey et al., 2004; Hughes et al., 1998). The European Fish Index (EFI+), the standard European fish-based index to estimate the ecological quality of aquatic ecosystems, follows this approach.

Both the IBI-derived and EFI+ indices integrate a group of fish metrics representative of ecological and biological characteristics of fish assemblages (Logez et al., 2013). In the case of EFI+, and although it is standardized to be applied across most type of European rivers and regions, including Spanish ones, its suitability varies from one site to another (Pont et al., 2007; Urbanič & Podgornik, 2008; Logez et al., 2010; Belmar et al., 2018). In the case of Spain, the country meets several conditions that often limit the performance of the EFI+ and hinder the development of a national-IBI index (Moyle & Marchetti, 1999; Ferreira et al., 2007; Segurado et al., 2014). First, rivers in Spain have low levels of alpha diversity (i.e. their rivers are species-poor) (Ferreira et al., 2007; Moyle & Marchetti, 1999), and thus richness metrics alone have low capability of differing between disturbed and undisturbed sites. Second, there is a high degree of endemicity in the Iberian Peninsula, with specific fish communities in every basin. Native species have adapted to resist extreme flow variations. Third, there is a lack of knowledge about ecological traits that hinder the development of trait-based metrics at a broad scale when many species are considered. Fourth, fish communities show an important intra- and inter- annual variability in their assemblage structure, caused by the natural annual variability of the hydrological and climatological patterns. Lastly, the lack of undisturbed sites makes the establishment of reference conditions more complex. For these reasons, several studies investigating, developing and testing metrics and indices at finer spatial scales, namely regions, basins and even single rivers, have been carried out in Spain (Ferreira et al., 2007; Segurado et al., 2008). These studies have been helpful in the selection of metrics for this study.

Aparicio et al. (2011) developed an IBI index for the Júcar River Basin that shows a high correlation with human disturbances such as flow regulation. For this reason, some of the metrics used in that study are useful for this study too. In general terms, fish assemblage health can be evaluated at three levels: individual, population and community (Moyle et al., 1998). A healthy fish individual should have a robust body without anomalies such as deteriorated fins and parasites and with a good growth rate. Metrics related to anomalies are commonly used in Spain (e.g. Aparicio et al., 2011). A healthy fish population is abundant and formed by multiple age classes, evincing reproductions, and viable habitat conditions for growth. Classical and basic metrics such as abundance and size (or age) structure are used at a population level (e.g. EFI+; Belmar et al., 2018). Finally, a healthy fish community consists of native species resilient to the specific extreme conditions at the site. Metrics related to alien species, such as relative number of alien individuals, are also used worldwide and are essential to be considered in the Iberian Peninsula, where alien species suppose a major threat to native fishes (García-Berthou et al., 2014).

As Spain is a poor-species fish region, the use of fish metrics based on biological and ecological traits is especially suggested for studying large regions. This is because biological communities from different geographical zones may vary in species composition but may have matching biological or ecological traits (Bonada et al., 2007). Traits are any feature that reflects species adaptation to their environment. They can be classified into biological traits, which describe life cycle, physiological, and or behavioral characteristics, and ecological traits, which are linked to habitat preferences, water flow, pollution, or temperature tolerances (Menezes et al., 2010). Traits that have been shown to have ecological relevance in Spain and other semi-arid regions are trophic specialization (e.g. Belmar et al., 2018), reproductive strategy (Colin et al., 2018), and life-history strategies, namely opportunistic, equilibrium or periodic species (Fornaroli et al., 2020).

Considering the Spanish particularities and the reviewed literature, the metrics for this study were selected following these considerations: (i) as this study consists of different stretches, and to allow river comparison, metrics that depend on specific environmental conditions and fish-specie characteristics must not be used. For instance, fish biomass (Colin et al., 2018) is not appropriate for this study as the different species that compose every river have different weight-specific characteristics; (ii) selected metrics must be representative of the three levels of fish assemblage health: individuals, community, and population; (iii) selected metrics must be a combination of both taxonomic community composition and ecological traits community composition metrics.

Table 5 gathers the 32 metrics that were studied and Table 4 the descriptions of the ecological traits used in this study. Ecological traits of trophic interaction and habitat use that were used to calculate the corresponding metrics were found in the database generated by Cano-Barbacil et al. (2020) for all the sampled species. Traits related to life history strategy were found in Fornaroli et al. (2020) for most of the sampled species. The sampled species that were not found there, namely *Cobitis calderoni, Iberochondrostoma lemmingii, Luciobarbus bocagei, Luciobarbus sclateri, Parachondrostoma miegii, Phoxinus bigerri, Pseudochondrostoma duriense, Salmo salar, Squalius pyrenaicus,* were determined following the indications in Fornaroli et al. (2020) based on body size, maturation time, fecundity per spawing time, juvenile survivorship, reproduction strategy.

Table 4. Description of characteristics used in the fish metrics

Alien	Not endemic to Spain
Translocated	Endemic to Spain but not the river
Opportunistic	Small-bodied species with earlymaturation and lowjuvenile survivorship and often are associated with habitats defined by frequent and intense disturbance, mirroring the classic r strategy
Periodic	medium to large body size, late maturation, high fecundity, and low juvenile survivorship and are likely to be favoured in highly periodic (seasonal) environments
Equilibrium	Small to medium in body size with intermediate times to maturity, low fecundity per spawning event, and high juvenile survivorship largely due to high parental care and small clutch size, closely aligned with the K strategy
Piscivorous	Feed mainly on other fishes.
Invertivores	Feed on invertebrates
Omnivorous	Consume considerable amounts of both plant and animal material.
Rheophilic	Preferring to live in running water.
Tolerant to habitat degradation	Have a large water quality and habitat flexibility.
Intolerant to habitat degradation	Have a low water quality and habitat flexibility.
Lithophilic spawning	Species that deposit eggs on a rock, rubble or gravel bottom where their embryos and larvae develop

Table 5. Biological metrics computed to characterize fish community

Acronym	Description	Mathematical expression	Reference
Richness			
RICHNESS	num of species	ns	EFI+
Abundance			
CPUE	num of individuals per unit of effort	n/surface	(Aparicio et al., 2011) (Belmar et al., 2018)
Origen			
RICHNESS_N	num of native species	ns_native	(Fornaroli et al., 2020)
RICHNESS _A	num of alien species, included translocated ones.	ns_alien	(Fornaroli et al., 2020) (Aparicio et al., 2011)
CPUE_N	num of native individuals per unit of effort	n_native/surface	
CPUE_A	num of alien individuals per unit of effort	n_alien/surface	(Aparicio et al., 2011) (Belmar et al., 2018)
ALIEN	num of alien individuals relative to the num of total individuals	n_alien/n	(Aparicio et al., 2011)
Size			
CPUE151	num of individuals >150 mm (total length) per unit of effort	n151/surface	EFI+
CPUE150	num of individuals <=150 mm (total length) per unit of effort	n150/surface	EFI+
Anomalies			
ANOM	num of individuals with anomalies relative to total individuals	n_anom/n	(Aparicio et al., 2011) (Belmar et al., 2018)
Life history strategies			
RICHNESS_OP	num of opportunistic species	ns_op	(Fornaroli et al., 2020)
RICHNESS_PE	num of periodic species	ns_pe	(Fornaroli et al., 2020)
RICHNESS_EQ	num of equilibrium species	ns_eq	(Fornaroli et al., 2020)
Trophic interaction			
RICHNESS_PIS	num of native piscivorous species	ns_pis	
RICHNESS_INV	num of invertivores species	ns_inv	
RICHNESS_OMN	num of omnivorous species	ns_omn	
CPUE_PIS	num of native piscivorous individuals per unit of effort	n_pis/surface	(Belmar et al., 2018)
CPUE_INV	num of invertivores individuals per unit of effort	n_inv/surface	(Belmar et al., 2018)

CPUE_OMN	num of omnivorous individuals per unit of effort	n_omn/surface	(Belmar et al., 2018) (Gartzia de Bikuña et al., 2017)
OMN	num of omnivorous individuals relative to total individuals	n_omn/n	(Belmar et al., 2018)
Habitat use			
RICHNESS_RHEO	num of rheophilic species degradation		(Aparicio et al., 2011)
CPUE_RHEO	num of rheophilic individuals per unit of effort		
RICHNESS_TH	num of species tolerant to habitat degradation	ns_th	(Gartzia de Bikuña et al., 2017)
CPUE_TH	num of individuals tolerant to habitat degradation per unit of effort		(Gartzia de Bikuña et al., 2017)
RICHNESS_IH	num of species intolerant to habitat degradation	ns_ih	
RICHNESS_THA	num of alien species tolerant to habitat degradation	ns_tha	(Belmar et al., 2018)
THN	num of native individuals tolerant to habitat degradation relative to total individuals	n_thn/n	(Belmar et al., 2018)
TH	num of individuals tolerant to habitat degradation relative to total individuals	n_th/n	
IH	num of individuals intolerant to habitat degradation relative to total individuals	n_ih/n	(Belmar et al., 2018)
CPUE_IH	num of individuals ≤150 mm (total length) intolerant to habitat degradation per unit of effort	n_ih/surface	
RICHNESS_LITHO	num of lithophilic spawning species	ns_litho	(Aparicio et al., 2011) (Belmar et al., 2018)
CPUE_LITHO	num of lithophilic spawning individuals per unit of effort	n_litho/surface	(Aparicio et al., 2011)

Note. Metrics without reference are decided to be tested by the author, based on similar metrics.

2.7. Reduction of metrics and study of eco-hydrological relationships

As both the fish and hydrological information were characterized by a large number of metrics, a reduction of the selected metrics was performed to simplify the following analysis of eco-hydrological relationships. Metrics that were highly correlated and did not have any statistically significant relationships were discarded.

Highly correlated metrics were excluded through a Principal Component Analysis (PCA), which is a statistic multivariate technique that often is used to reduce the dimensionality of large data sets and remove redundant metrics. It calculates the eigenvectors of every metric from the covariance matrix of the standardized data to generate as many principal dimensions as needed to represent the variance of the sample. A PCA was performed for both fish and hydrological metrics independently. and the metrics that explained greater variance of the sample in the main principal dimensions were selected. To select the principal dimensions (also called components) that explained the greatest variance of the sample, the proportion of explained variance accumulated by the principal dimensions was evaluated, and the minimum number of principal dimensions from which the next accumulated explained variance was no longer substantial were selected. Then, metrics that were most strongly correlated with each principal dimension (> 0.6 or longer eigenvectors vectors in either direction in the PCA graphs) were selected, assuming that they provided more information of the sample. Finally, highly correlated metrics (parallel vectors in the PCA graphs) were identified.

Statistically significant relationships were evaluated for every pair of fish and hydrological metrics mainly by Pearson correlations. The Pearson correlation coefficient (r) and the significance coefficient (p) were calculated. The Pearson correlation coefficient gives information about the linear relationship between two metrics. The significance coefficient quantifies to what extent a correlation result can be due to random variation in the included metrics, or due to some factor of interest. This analysis complemented and supported the selection of metrics based on their role in the PCA. This is to say, if two or more metrics were highly correlated among them in the PCA, the metric that showed higher correlation and significance with fish metrics in the correlation analysis was selected. In addition, metrics explaining low correlation with the main principal components of the PCA but showing relevant eco-hydrological relationships were included too. This method ensured the inclusion of metrics relevant for both the PCA and the correlation analysis, while preventing from discarding metrics that showed relevant eco-hydrological information. Metrics that were zero for most of the rivers were in general discarded too.

Once the final fish and hydrological metrics were selected, their relationships were analyzed through visual observation of scatter plots, linear Pearson correlation graphs, and hierarchical cluster analyses based on Pearson correlation coefficient. Linear correlations were commonly used for the study biological responses to flow regimes and is also appropriate for low size sample.

2.8. Potential limitations with data and analytical methods

This study had two important data limitations that constrained the study of eco-hydrological relationships. First, pre-dam hydrological records were not available, and thus the hydrological alteration of each river could not be estimated, nor associated to biological metrics. Second, the fact that there was only fish data available for a single sampling prevented this study from analyzing changes in the fish community of each river across time. Consequently, the method used in this study could not contemplate any kind of comparison within the same river as some methodologies suggest (e.g. the ELOHA method (Poff et al., 2010)), that evaluate fish metrics throughout a gradient of hydrological alteration or fish metrics in response to flow conditions varied in time. Regardless the single fish sampling, to study associations between long term hydrological records and a unique fish sampling can provide relevant information, assuming that fishes are representative of long- and short-term hydrological conditions.

The implemented method, based on the study of eco-hydrological relationships by linear Pearson correlation, is useful to identify linear relationships between fish and hydrological metrics, but it is not able to identify non-linear relationships. In addition, the low size of the sample (33 observations), could result in false high correlation coefficients if there is one or several observations in the sample that are outliers. For this reason, visual inspection of the scatter plots was important to detect nonlinear relationships and outliers. Another way to address this limitation, and to ensure the plausibility of the resulted eco-hydrological relationships, was to complement and based the results on known ecological links between flow and fishes. This was done in the discussion part.

Finally, it is worthy to be mentioned that this study should be understood as a preliminary analysis developed with the information that was available at the moment of the author's internship.

3. Results

3.1. Reduction of the dimensionality of hydrological metrics

The initial 40 hydrological metrics were reduced to 9 by a PCA (Figure 8). The first four principal dimensions explained 70.5% of the variance (Figure 9). Figure 10 helped to explore the outputs of the PCA functions, seeing which metrics were more correlated with the first four dimensions.

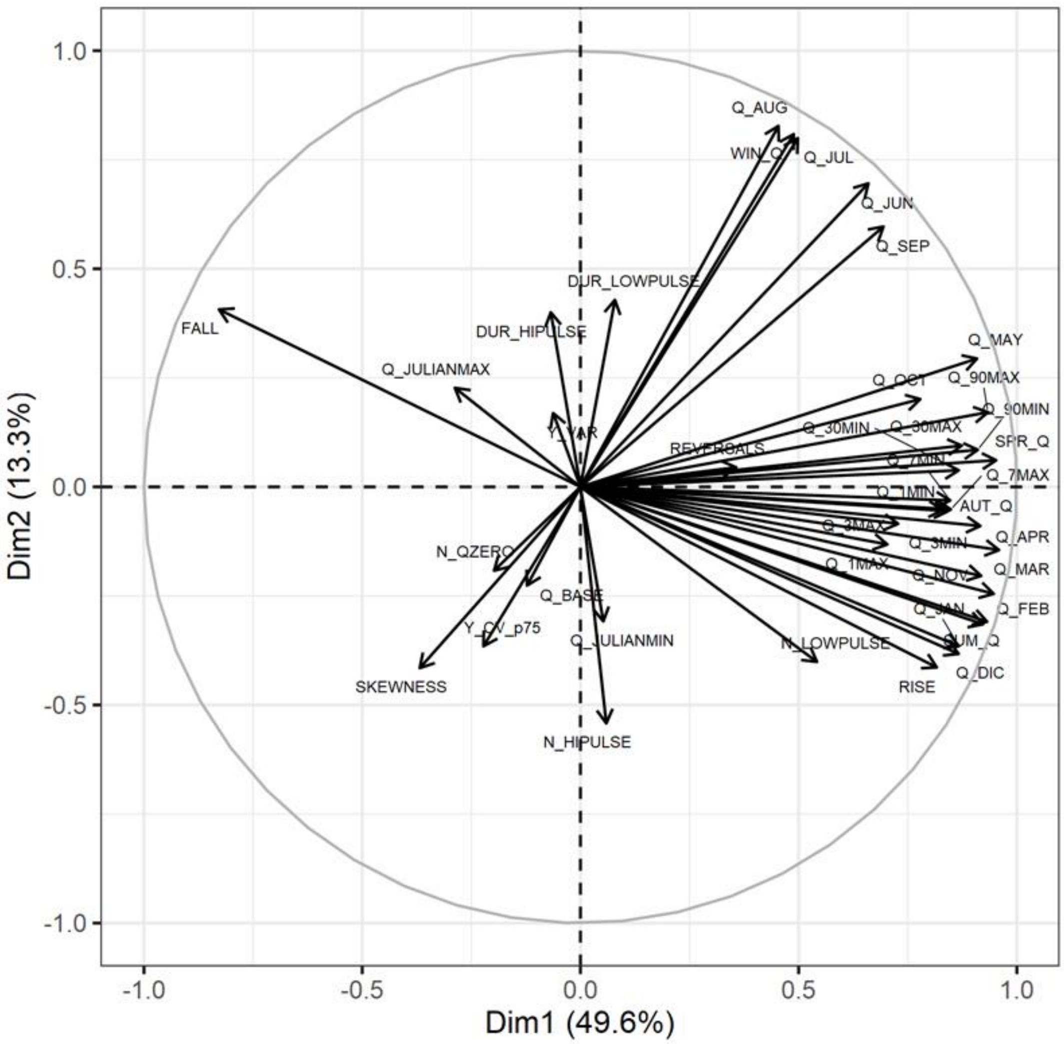


Figure 8. PCA of the 40 initial hydrological metrics

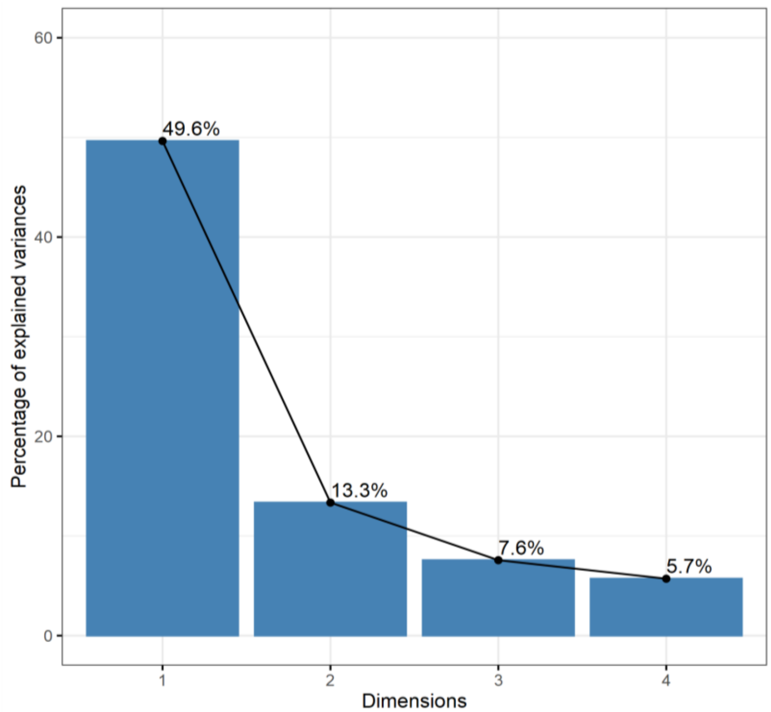


Figure 9. Contribution of every dimension to the explained variance of the sample represented by the initial 40 hydrological metrics.

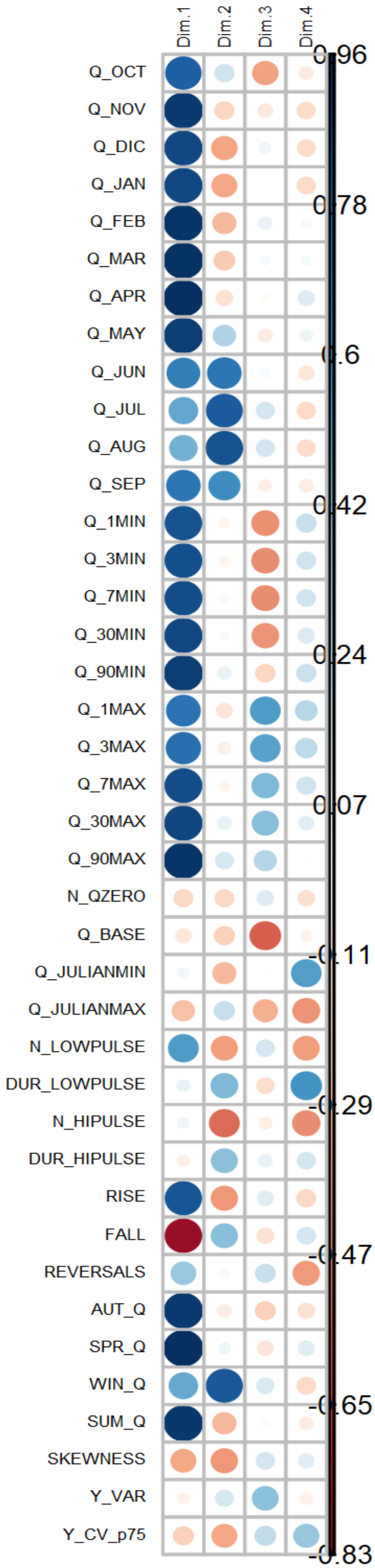


Figure 10. Results of the PCA for the initial 40 hydrological metrics. Degree of correlation between metrics and dimensions (size of the circle) and correlation sign (color).

Magnitude of monthly and seasonal flows showed high internal correlation (Figure 8) and similar values of correlation with the main principal dimensions in the PCA (Figure 9). The flow magnitude in February, March and April and the flow magnitude in spring and summer showed correlations higher than 35% in the correlation analysis. They also exhibited sensitivity to the same biological metrics (Figure 11). The rest of the monthly and seasonal metrics showed a lower correlation. The flow magnitude in April and spring were selected for the study of eco-hydrological relationships, as they presented higher correlation values.

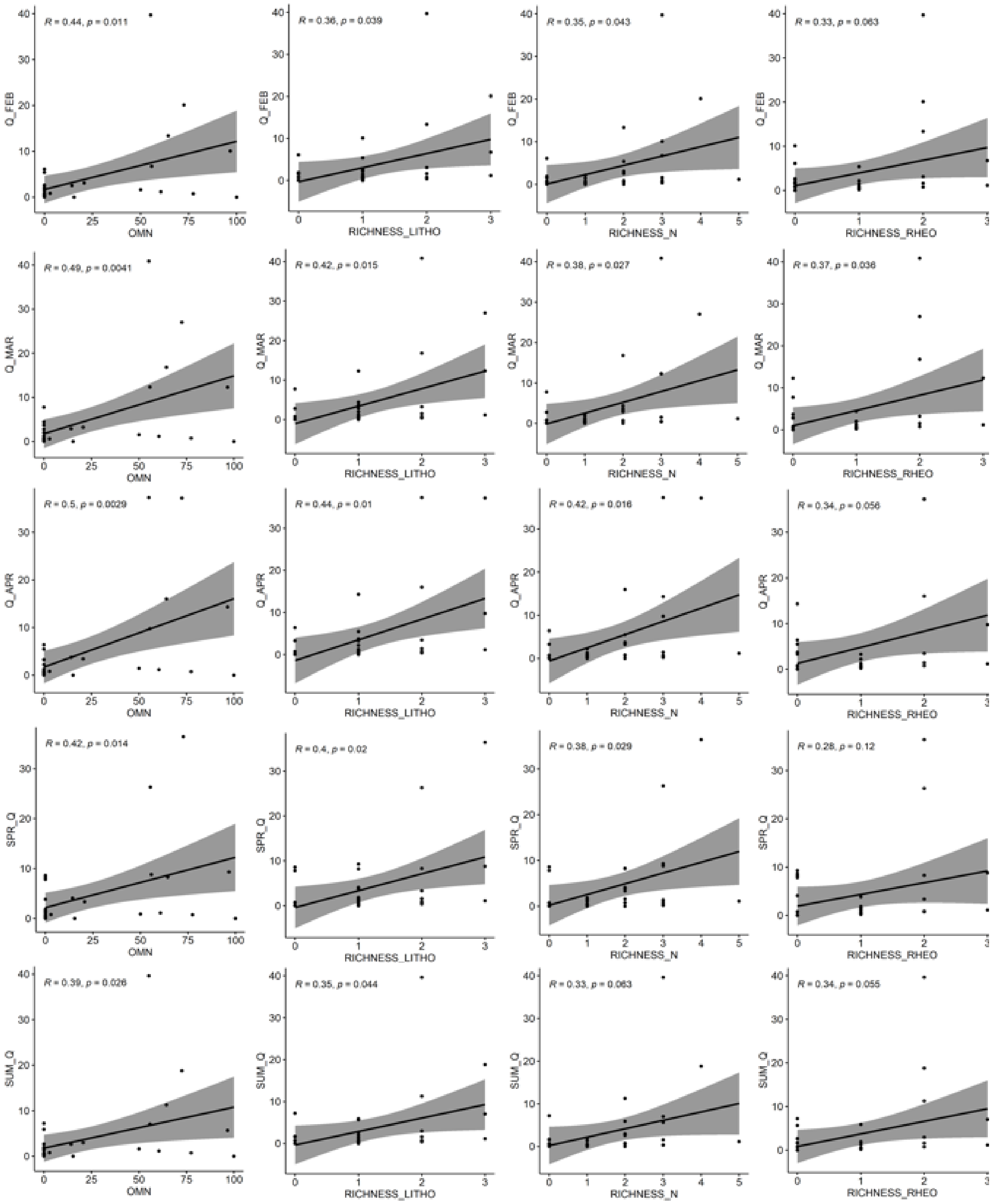


Figure 11. Correlation graphs for the hydrological metrics of magnitude (flow in February (Q_FEB), flow in March (Q_MAR), flow in April (Q_APR), spring flow (SPR_Q) and summer flow (SUM_Q) with the fish metrics of proportion of omnivorous individuals (OMN), richness of lithophilic spawning species (RICHNESS_LITHO), richness of native species (RICHNESS_N) and richness of rheophilic species (RICHNESS_RHEO). All of them had similar positive correlation with the same fish metrics, where Q_APR showed the highest correlation values with fish metrics.

Metrics of magnitude and duration of annual extreme flow conditions were also internally correlated with magnitude of monthly and seasonal flows metrics in the PCA (Figure 8) and showed associations to the same fish metrics. Metrics of moving annual maximum flows presented slightly higher correlation with fish metrics, although metrics of moving annual minimum flows also were correlated with some fish metrics. As all of them responded, in general, to the same fish metrics, the annual maximum flow of 1 day and the annual minimum flow of 1 day were selected for the study of eco-hydrological relationships because they presented higher correlation values.

The metrics number of low and high pulses within each water year, duration of low and high pulses, rise rate and fall rate, variability across annual flows and Julian date of annual minimum flow of 1 day showed low correlation with fish metrics and were discarded. The metric number of zero-flow days was also excluded from the analysis, as it had almost no variability for the sample, as most of the values were zero. Lastly, even though the base flow showed low correlation with principal dimensions (Figure 8) and low correlation with most of the fish metrics, it was included in the study, because it showed association with the fish metric proportion of individuals intolerant to habitat degradation (Table 8). Table 6 presents the excluded fish metrics and their reasons for exclusion. For the selected 9 hydrological metrics (Figure 12), the fist four principal dimensions explained a variance of the sample of 86.2% (Figure 13).

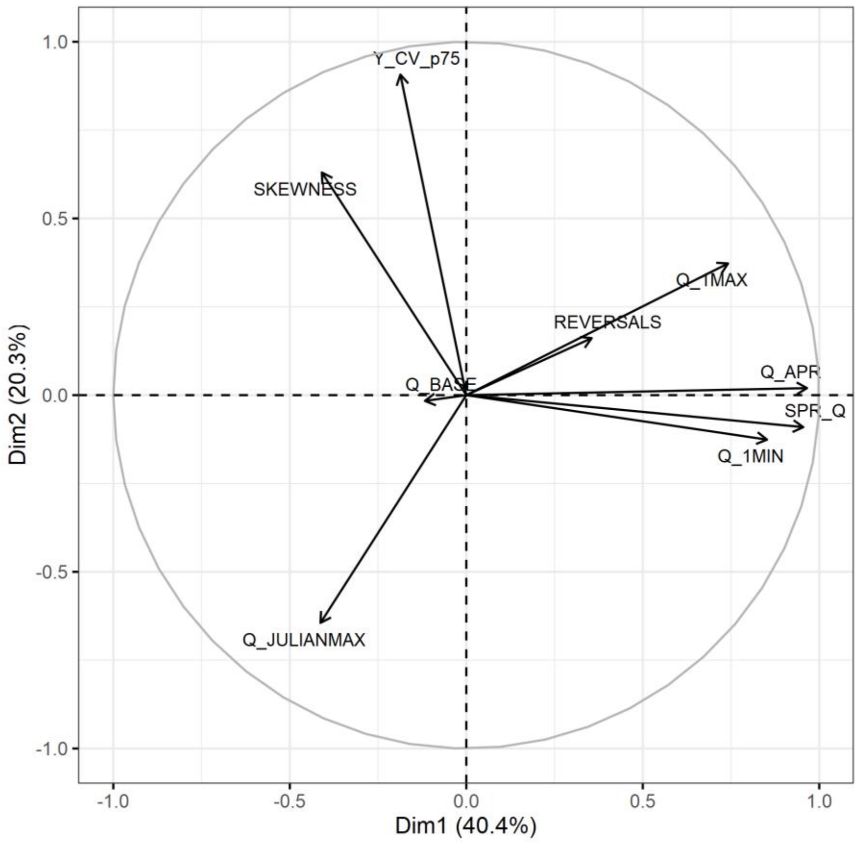


Figure 12. PCA of the selected 9 hydrological metrics included in the study of eco-hydrological relationships.

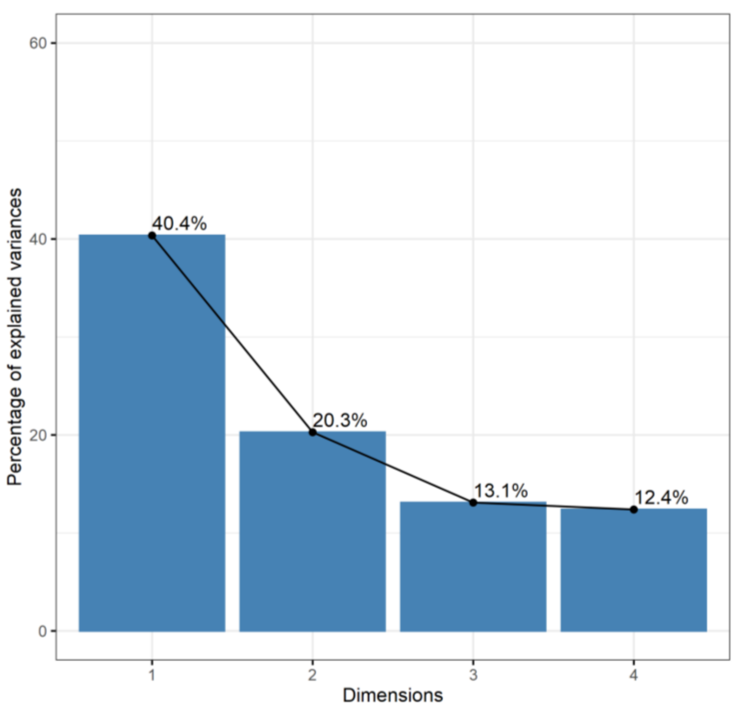


Figure 13. Contribution of every dimension of the PCA to the explained variance of the sample represented by the selected 9 hydrological metrics

Table 6. Excluded hydrological metrics and cause of exclusion.

Metric	Cause of exclusion
Group 1: *Magnitude of monthly and seasonal water conditions*	
Q_OCT, Q_NOV, Q_DIC, Q_JAN, Q_FEB, Q_MAR, Q_MAY, Q_JUN, Q_JUL, Q_AUG, Q_SEP	Highly correlated with Q_APR Similar relationships with fish metrics
AUT_Q, WIN_Q, SUM_Q	Highly correlated with Q_APR Similar relationships with fish metrics
Group 2: *Magnitude and duration of annual extreme flow conditions*	
Q_3MIN, Q_7MIN, Q_30MIN, Q_90MIN, Q_3MAX, Q_7MAX, Q_30MAX, Q_90MAX	Highly correlated with Q_1MIN and Q_1MAX Similar relationship with fish metrics
N_QZERO	Many values were zero
Group 3: *Timing of annual extreme flow conditions*	
Q_JULIANMIN	No relationship with fish metrics
Group 4*: Frequency and duration of high and low pulses*	
N_LOWPULSE, N_HIPULSE	No relationship with fish metrics
DUR_LOWPULSE, DUR_HIPULSE	No relationship with fish metrics
Group 5: *Rate and frequency of water condition changes*	
RISE, FALL	No relationship with fish metrics
Y_VAR	No relationship with fish metrics

3.2. Reduction of the dimensionality of fish metrics

The same procedure was followed for selecting informative and ecologically relevant fish metrics. The initial 32 fish metrics were reduced to 13 by a PCA (Figure 14). The first 4 principal dimensions explained 72.8% of the variance (Figure 15). Figure 16 helped to explore the outputs of the PCA functions, seeing which metrics were more correlated with the first four dimensions.

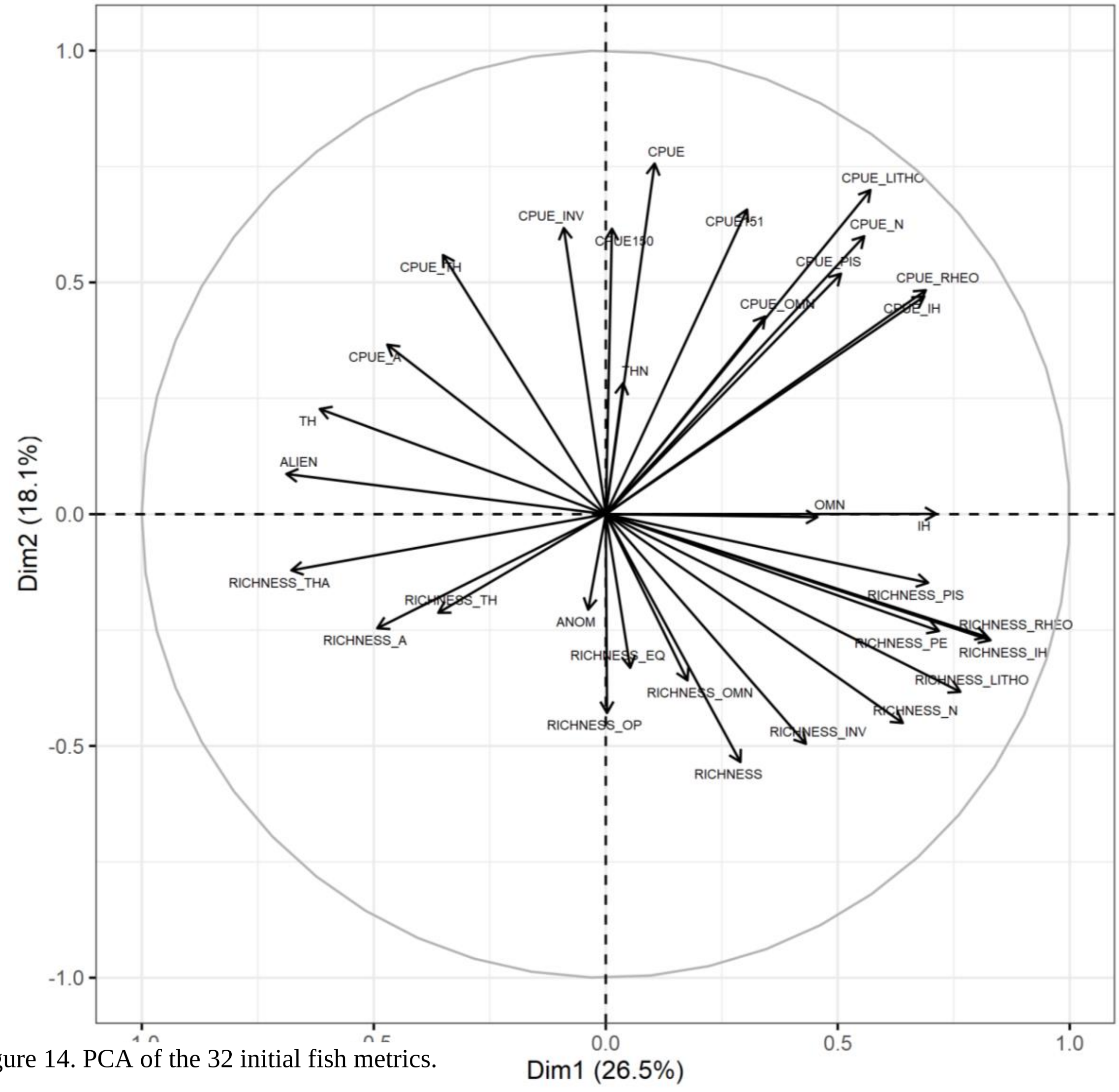
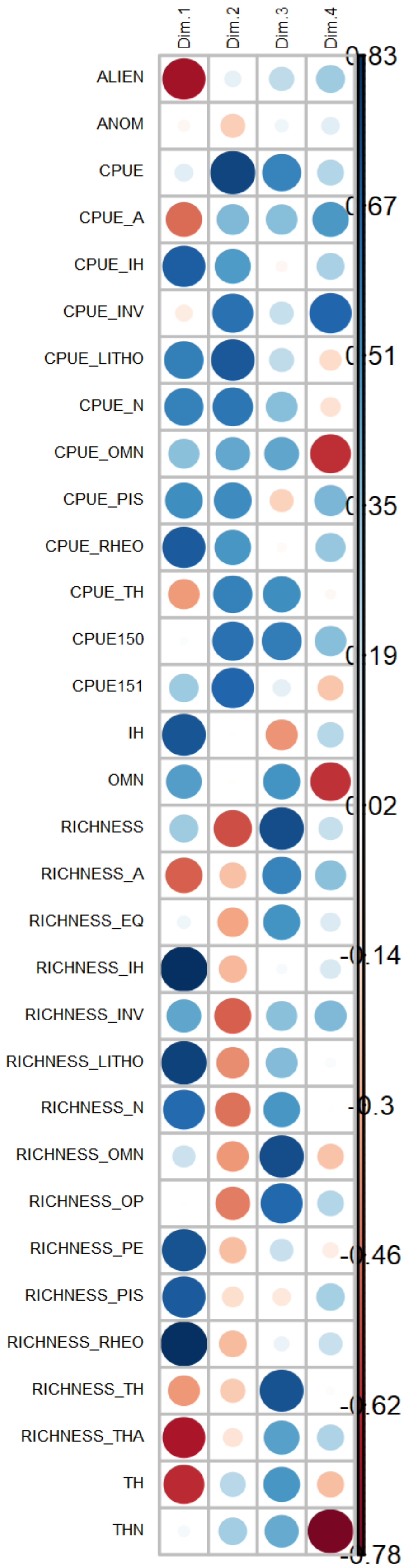


Figure 14. PCA of the 32 initial fish metrics.



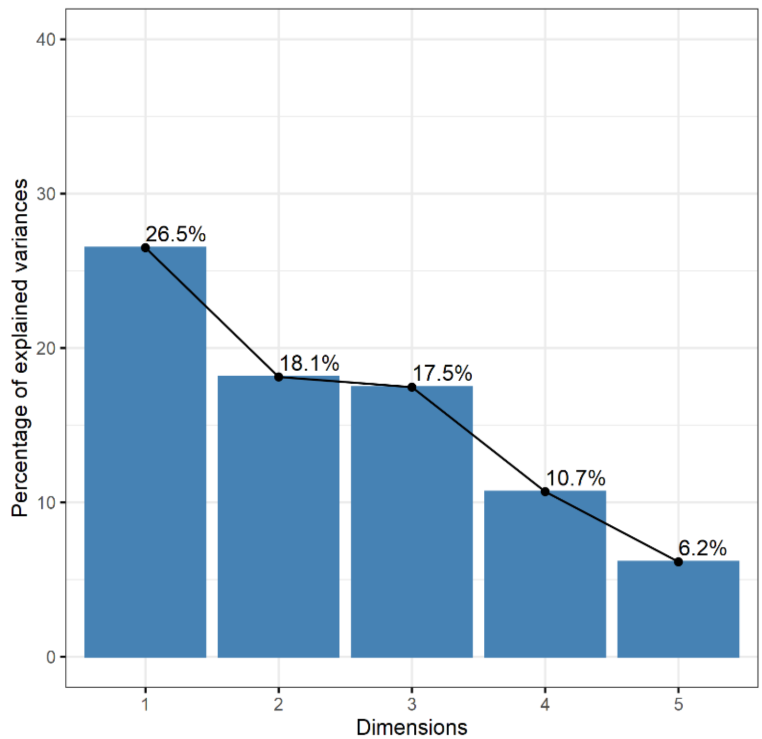


Figure 15. Contribution of every dimension of the PCA to the explained variance of the sample represented by the initial 32 fish metrics

Figure 16. Results of the PCA for the initial 32 fish metrics. Degree of correlation between metrics and dimensions (size of the circle) and sign (color).

The correlation and cluster analysis for every pair of biological and hydrological metrics helped to study relationship. See example of the correlation analysis in Figure 17 and Figure 18. Metrics showing low correlation (lower than 0.6) with the main principal dimensions and not showing fish-flow relationships were excluded. Table 7 presents the excluded fish metrics and their reasons for exclusion. The metric proportion of omnivorous was kept, even though it was high correlated with the metric IH in the PCA, because it showed high correlation with magnitude metrics (see Pearson correlation coefficient for this metric in Table 8).

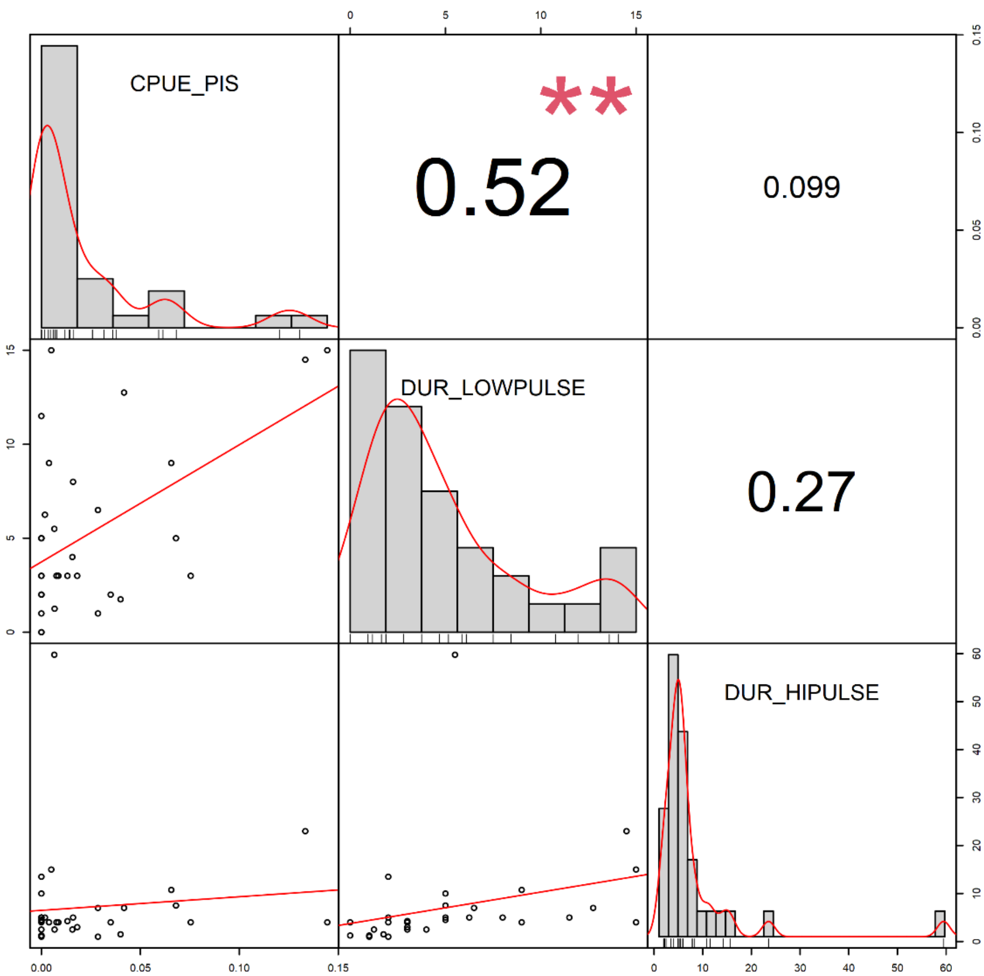


Figure 17. Correlation analysis for the fish metric abundance of piscicolas individuals (CPUE_PIS) with two hydrological metric of duration of low and high pulses (DUR_LOWPULSE and DUR_HIPULSE).

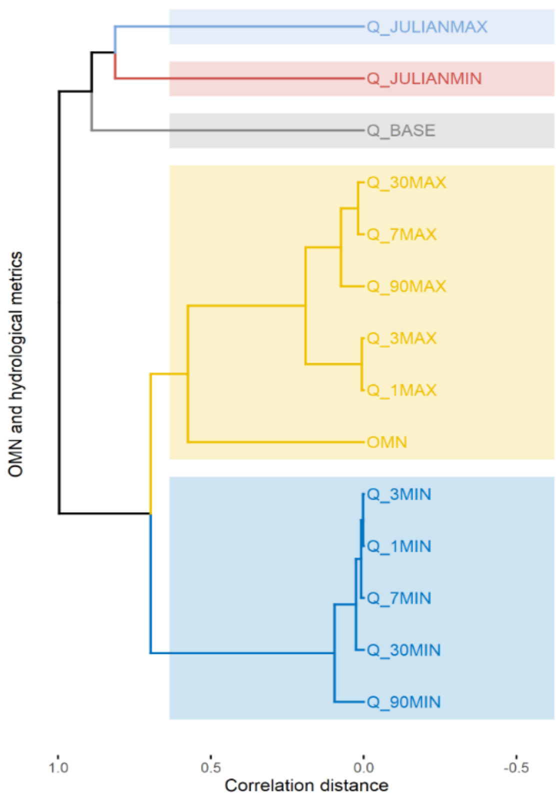


Figure 18. Example of cluster analysis based on correlation coefficients for the fish metric proportion of omnivorous individuals (OMN). Every color is a cluster. OMN is in the same cluster than the moving annual maximum flows metrics (Q_1MAX, Q_3MAX, Q_7MAX, Q_30MAX, Q_90MAX)

Table 7. Excluded fish metrics and cause of exclusion.

Metric	Cause of exclusion
ANOM	Little correlation with PC No relationship with hydrological metrics
CPUE_IH	Highly correlated with CPUE_RHEO
CPUE_INV	No relationship with hydrological metrics
CPUE_OMN	Highly correlated with CPUE_LITHO
CPUE_PIS	No relationship with hydrological metrics
CPUE_TH	No relationship with hydrological metrics
RICHNESS_EQ	Little correlation with PC
RICHNESS_IH	Highly correlated with RICHNESS_RHEO
RICHNESS_INV	Highly correlated with RICHNESS_N
RICHNESS_OP	Little correlation with PC No relationship with hydrological metrics
RICHNESS_PE	Little correlation with PC No relationship with hydrological metrics
RICHNESS_PIS	Little correlation with PC Not relevant correlation with hydrological metrics
RICHNESS_TH	Little correlation with PC
RICHNESS_THA	No relationship with hydrological metrics
THN	Little correlation with PC

PC=Principal component

In the final selection of the 13 fish metrics (Figure 19), the first three principal dimensions explained 72.6% of the sample variance by the first three dimensions (Figure 20).

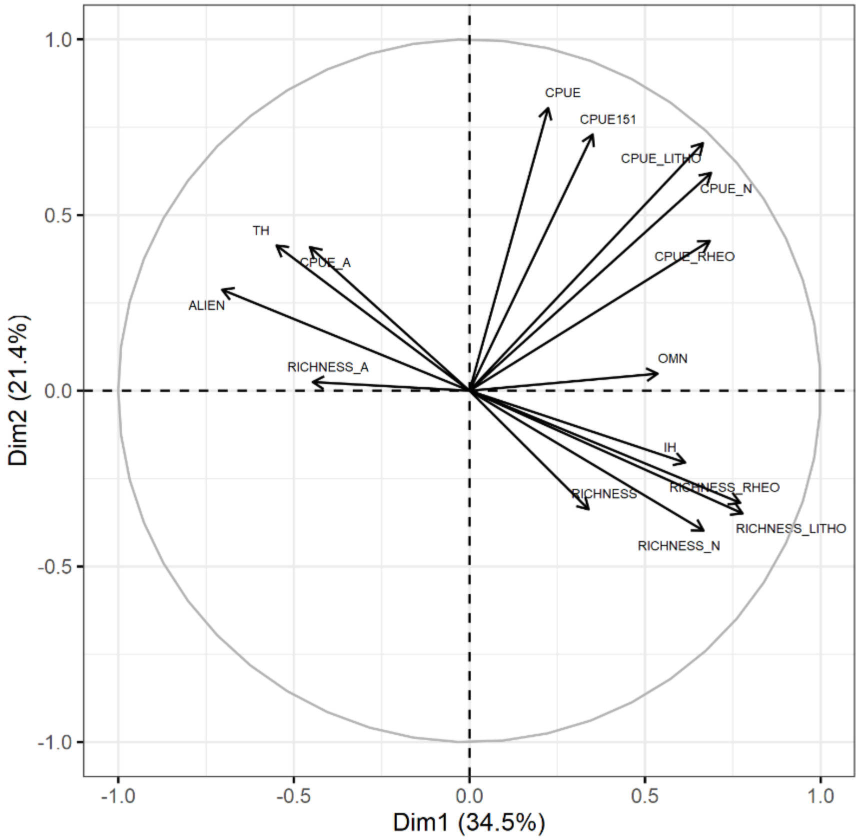


Figure 19. PCA of the selected 13 fish metrics.

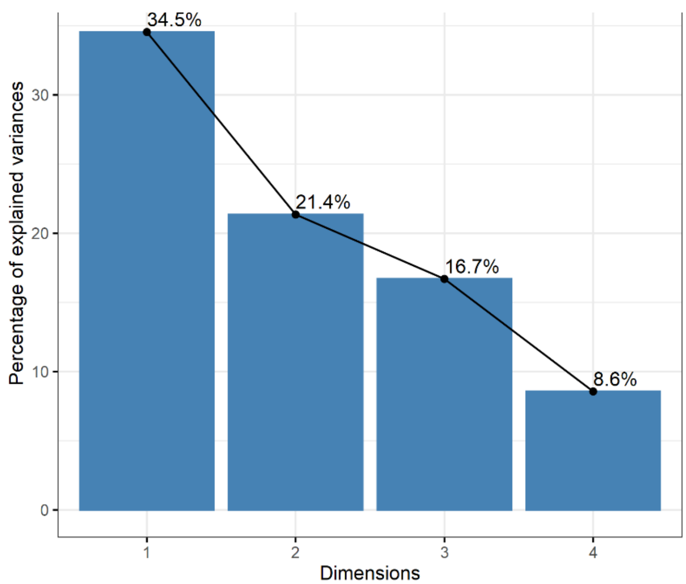


Figure 20. Contribution of every dimension of the PCA to the explained variance of the sample represented by the selected 13 fish metrics.

3.3. Eco-hydrological relationships

The correlation coefficients obtained for every pair of fish-flow metrics included for the analysis of eco-hydrological relationships are displayed in Table 8. The correlation coefficients were in general low, ranging from 0.003 to 0.622. Correlation coefficients higher than 0.34 and significances lower than 0.05 were the thresholds to consider fish-flow relationships ecologically relevant. In addition to the correlation coefficient and the statistical significance, scatter plots were used to evaluate non-linear tendencies. In total, 27 (2,11%) of the 1280 evaluated relationships (40 hydrological metrics x 32 fish metrics) had statistically interesting associations to produce ecological conclusions. These relationships are explained below.

Table 8. Pearson correlation coefficients for the selected fish and hydrological metrics. Correlations greater than 34% and significance lower than 0.05 are in green (positive correlation) and yellow (negative correlation) backgrounds

	Q_APR	**SPR_Q**	**Q_1MIN**	**Q_1MAX**	**Q_BASE**	**Q_JULIANMAX**	**REVERSALS**	**SKEWNESS**	**Y_CV_p75**
ALIEN	-0,23	-0.24	-0,20	-0,23	-0,05	0,34*	0,03	0,16	-0,08
CPUE	-0,21	-0.23	-0,16	-0,19	0,23	-0,25	-0,09	**0,40**	**0,39**
CPUE_LITHO	-0,16	-0.18	-0,13	-0,16	0,06	-0,24	**-0,47**	**0,42**	0,29
CPUE_N	-0,14	-0.16	-0,11	-0,12	0,08	-0,29	-0,33	**0,45**	**0,37**
CPUE_RHEO	-0,12	-0.14	-0,05	-0,15	0,24	-0,29	**-0,37**	0,15	0,20
CPUE151	-0,22	-0.24	-0,22	-0,20	-0,17	-0,21	**-0,36**	**0,35**	0,12
IH	0,12	0.054	0,23	-0,03	**0,43**	-0,20	-0,18	-0,06	0,07
OMN	**0,50**	**0.42**	**0,35**	**0,60**	-0,13	-0,39*	-0,06	0,20	0,16
RICHNESS	0,27	0.24	0,26	0,28	-0,03	**-0,45**	0,19	-0,14	0,05
RICHNESS_LITHO	**0,44**	**0.40**	**0,49**	0,29	0,10	**-0,55**	-0,09	-0,09	0,14
RICHNESS_N	**0,42**	**0.38**	**0,37**	**0,46**	-0,07	**-0,62**	0,13	-0,12	0,14
RICHNESS_RHEO	0,34	**0.28**	**0,40**	0,17	0,19	**-0,50**	-0,07	-0,06	0,12
TH	-0,12	-0.16	-0,23	0,05	-0,18	0,31	0,15	**0,43**	0,00

**Value of Pearson coefficient affected by outliers.*

All richness metrics were positively correlated with magnitude and duration metrics (Table 8 and Figure 21), and negatively or poorly correlated with duration, timing, frequency, and rate of change metrics (Table 8). This could implicate that richness is higher in larger rivers.

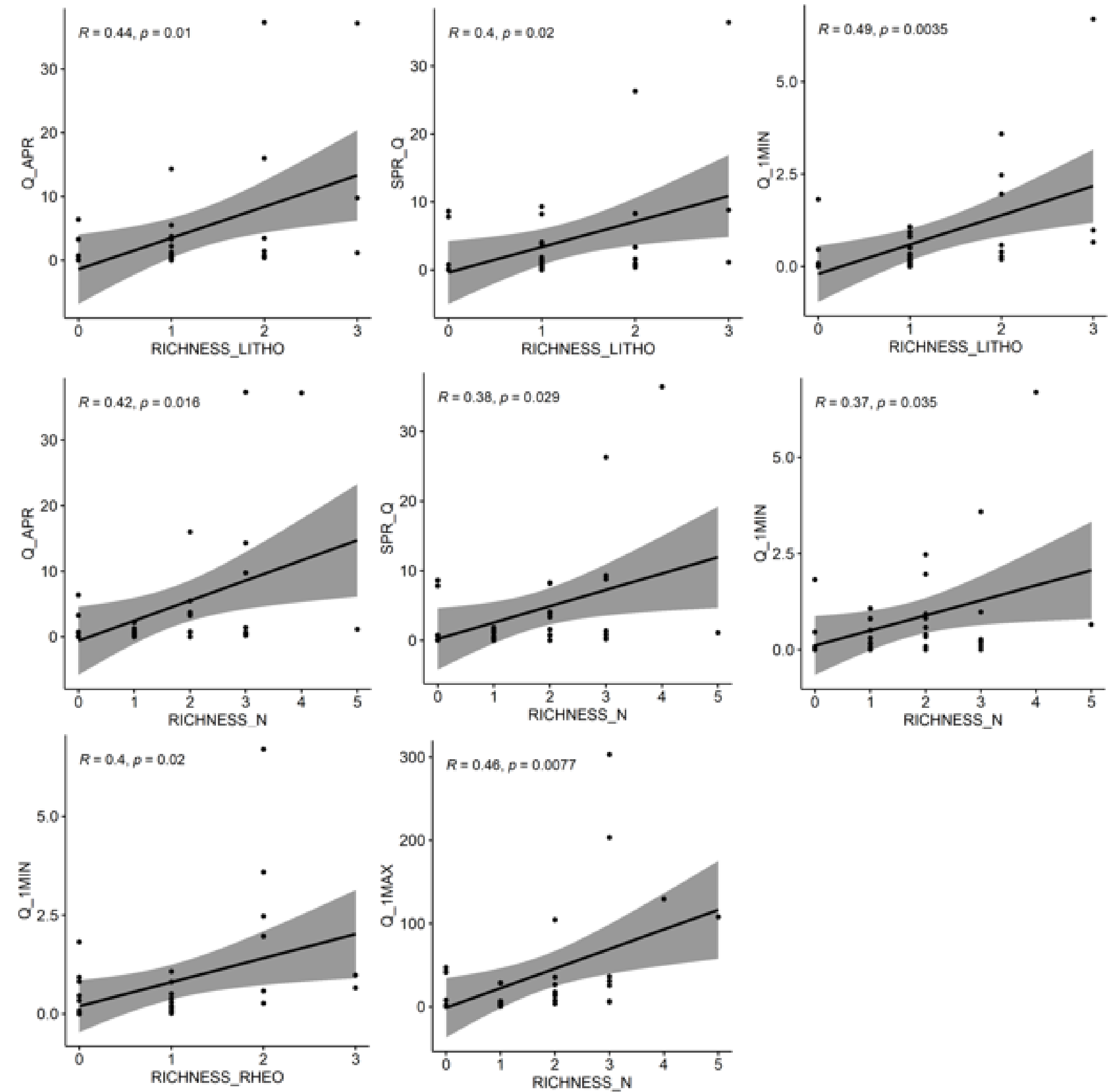


Figure 21. Correlation graphs of the richness metrics with magnitude and duration metrics. Richness of lithophilic spawning species = RICHNESS_LITHO; richness of native species = RICHNESS_N and Richness of rheophilic species = RICHNESS_RHEO.

All abundance metrics were negatively and poorly correlated with magnitude metrics (Table 8) and correlated with rate of change metrics, but unevenly so (Figure 22). On the one hand, all of them were positively correlated with skewness and variation of the annual flows higher than the percentile 75, where the latter had a lower correlation. A skewed hydrograph presents high differences between the annual maxima flow and the annual mean flow, while a non-skewed hydrograph tends to flow homogenization. The abundance of native individuals presented the highest positive correlation for these two metrics. On the other hand, all of them were negatively correlated with number of reversals. Reversals is the succession of frequent rises and falls. Thus, abundance in the studied rivers was higher as the skewness increases and lower as the number of reversals increase.

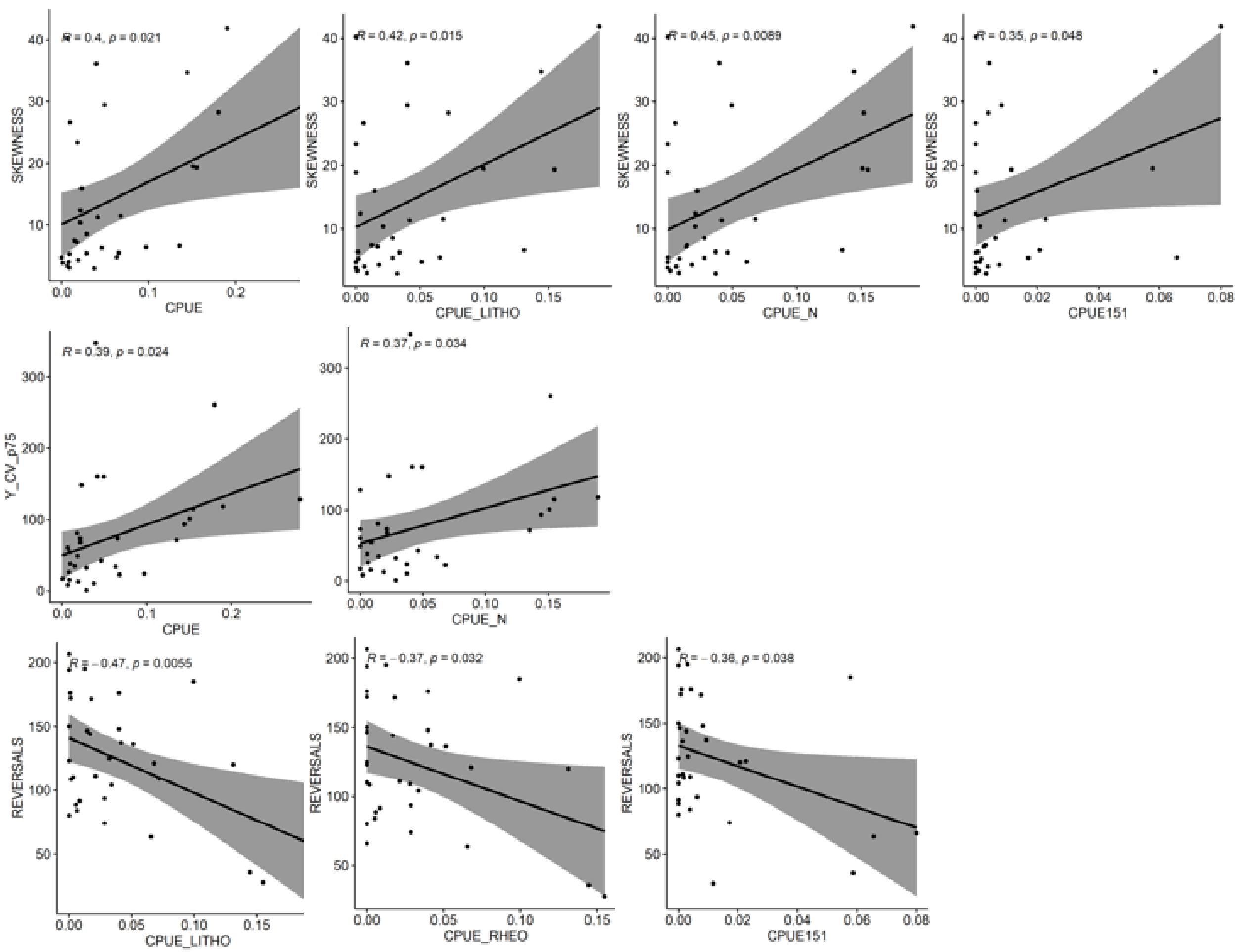
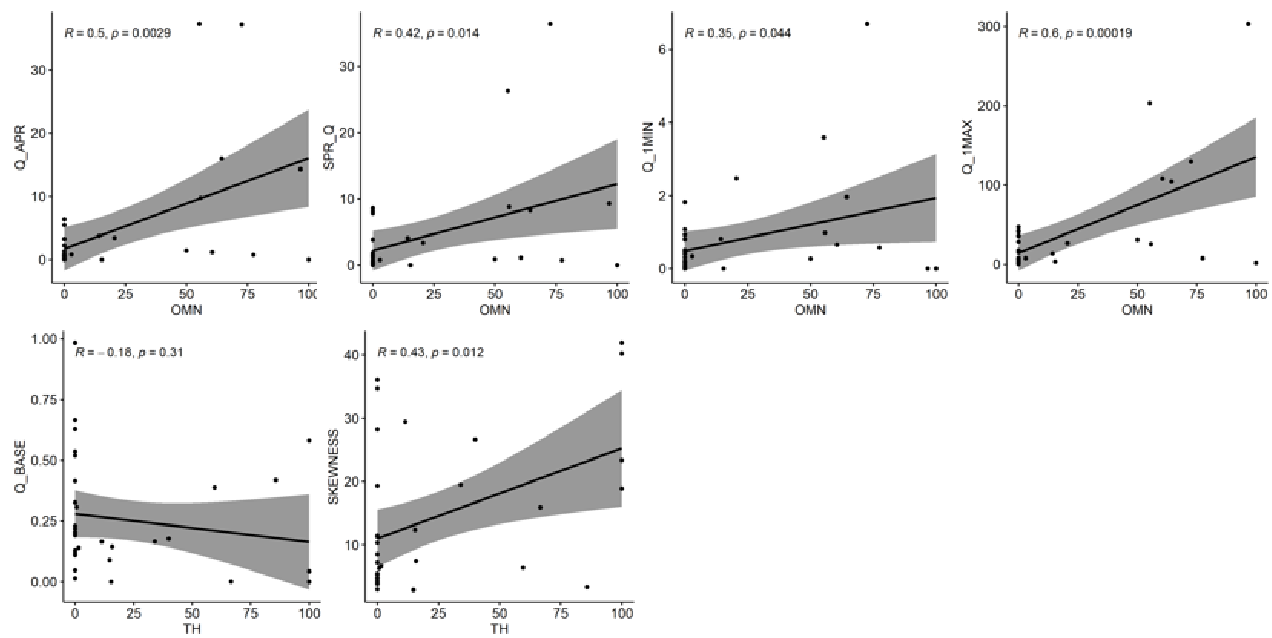


Figure 22. Correlation graphs of the abundance metrics with rate of change metrics. Abundance metrics were positively correlated with skewness (SKEWNESS) and variation of the annual flows higher than the percentile 75 (Y_CV_p75), and negatively correlated with number of reversals (REVERSALS). Abundance = CPUE; abundance of lithophilic spawning individuals = CPUE_LITHO; abundance of native individuals = CPUE_N; abundance of individuals larger than 150 mm = CPUE_151 and abundance of rheophilic individuals = CPUE_RHEO

Metrics of proportion, namely proportion of individuals tolerant or intolerant to habitat degradation and proportion of omnivorous individuals followed no common patterns of correlation with the hydrological metrics (Figure 23). Proportion of omnivorous individuals showed positive and high correlation with daily flow in April and annual maximum flow, potentially indicating that more larger rivers have a higher proportion of omnivorous individuals. Proportion of individuals intolerant to habitat degradation was positively related with base flow, indicating tendency of individuals intolerant to habitat degradation to live in rivers with a constant minimum flow. A river with a low base flow is a river with minimum flows much smaller in magnitude than median flows. In contrast, a river with a base flow equal 1 (assuming that the 7-day minimum flow is never higher than the median flow), is a river with minimum flows similar in magnitude to median flows, thus, a river with no variability in minimum flows. Proportion of individuals tolerant to habitat degradation was positively correlated with skewness. It is also noteworthy that, in contrast to individuals intolerant to habitat degradation, individuals tolerant to habitat degradation is negatively correlated with base flow, even though the correlation value is not high (r = - 0.18, Table 8 and Figure 23). Consequently, this could implicate that species tolerant to habitat degradation thrive in rivers with variability of both, high and low flows.



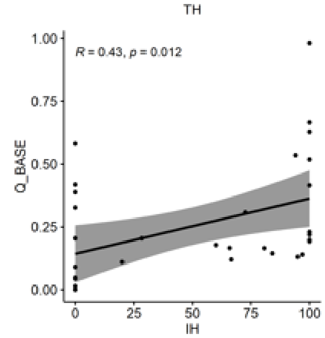


Figure 23. Correlation graphs of proportion metrics with hydrological metrics. Proportion of omnivorous individuals (OMN) was positively correlated with hydrological metrics of magnitude (flow in April (Q_APR), flow in spring (SPR_Q), annual minimum flow of 1 day (Q_1MIN) and annual maximum flow of 1 day (Q_1MAX)). Proportion of individuals tolerant to habitat degradation was positively correlated with skewness (SKEWNESS) and negatively and lowly correlated with base flow (Q_BASE). Proportion of individuals intolerant to habitat degradation was positively correlated with base flow (Q_BASE).

The Julian day of annual maximum flow, which was the only timing metric that was selected, was negatively correlated with all the fish metrics and presented high values of correlation especially for richness metrics (Figure 24). This may implicate that the timing of peak flows influences the richness of species in a river, in the way that rivers whose peak flow occur in autumn or winter tend to have a lower richness, whereas rivers whose peak flow occur in spring, tend to have a higher richness.

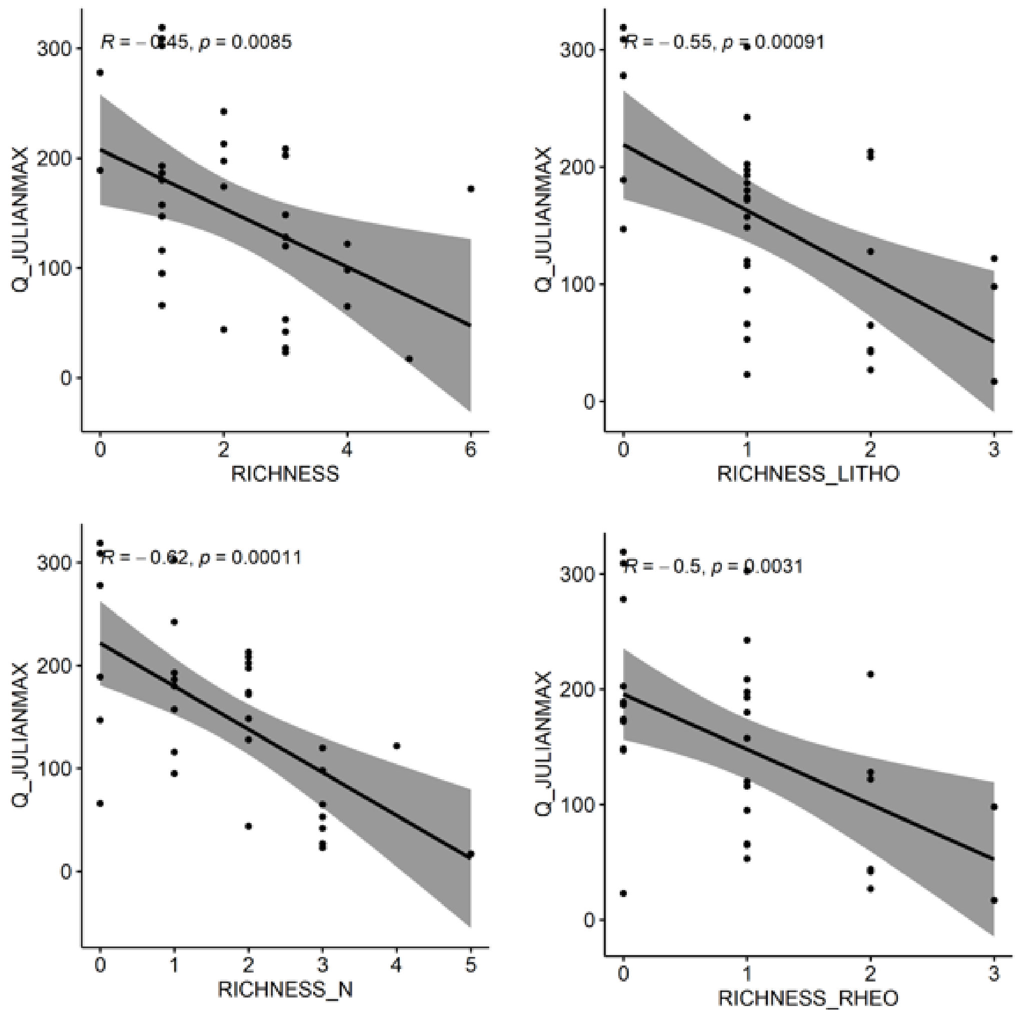


Figure 24. Correlation graphs of the metric julian day of annual maximum flow (Q_JULIANMAX) with fish metrics of richness (Richness = RICHNESS; Richness of lithophilic spawning species = RICHNESS_LITHO; richness of native species = RICHNESS_N and Richness of rheophilic species = RICHNESS_RHEO.)

Finally, the proportion of alien individuals was not highly correlated with hydrological metrics. However, it was possible to observe in the correlation graphs (Figure 25(b)) that rivers with lower flows contained higher proportions of alien individuals, whereas larger rivers presents zero or low proportions of alien individuals. Given the high number of rivers with zero alien individuals, a second correlation was conducted excluding rivers without zero alien individuals (Figure 25). The result of this analysis showed that the proportion of alien individuals was negatively associated with flow magnitude, showing the lower the streamflow the higher the density of alien individuals.

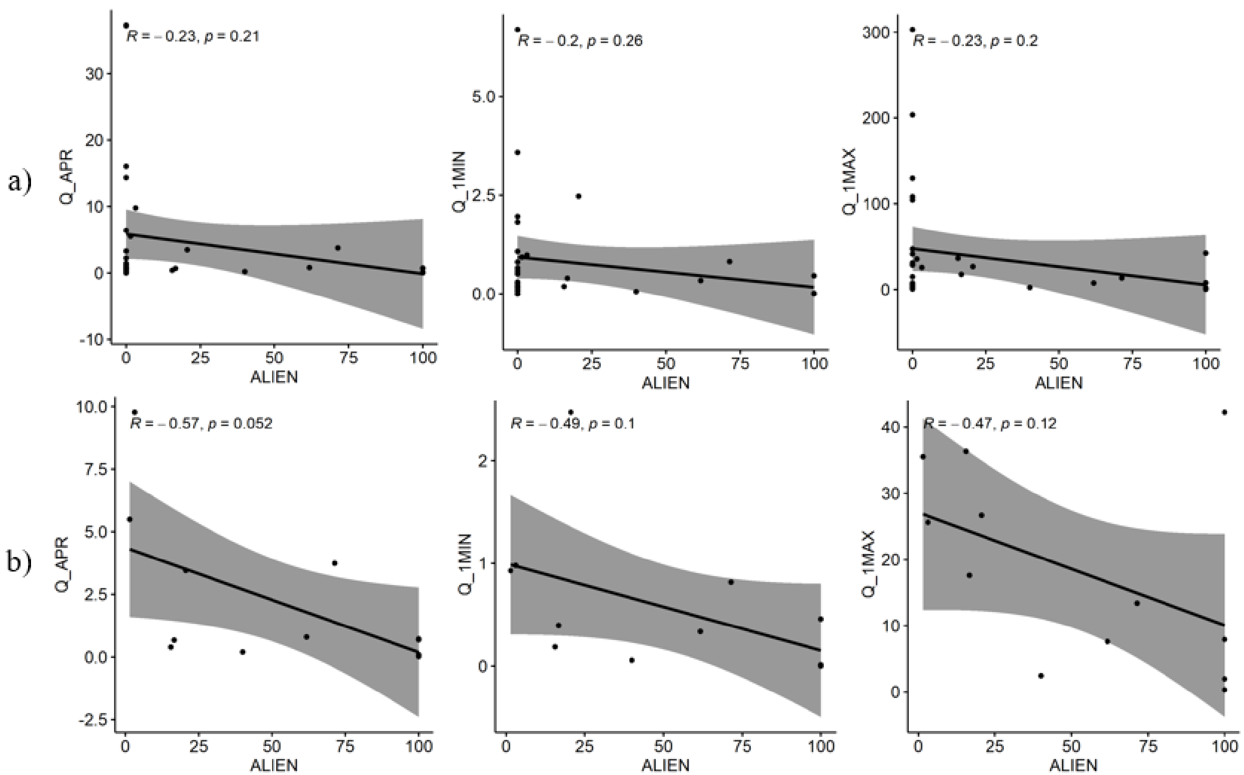


Figure 25. Correlation analysis for the fish metric proportion of alien individuals (ALIEN) with the hydrological metrics of daily flow in April (Q_APR), annual maximum flow (Q_1MIN) and annual minimum flow (Q_1MAX). The three correlations on the top (a) are computed with the entire number of rivers, whereas the three correlations at the bottom (b) exclude those rivers whose ALIEN values equal zero. Rivers with lower flows tend to have higher proportion of alien individuals.

4. Discussion

This section discusses the potential biological implications of the statistical relationships that were found and puts the results of this study into context with other findings in the reviewed literature. Limitations with the study are also included to explain the obtained results.

Richness metrics had positive correlation with magnitude metrics, whereas abundance metrics responded negatively. This indicates that rivers with higher flows tend to have a greater fish diversity than rivers with lower flows. This concurs with the idea that higher flows imply greater habitat volume, which generates more heterogeneity of mesohabitats and potential habitats for species with different ecological traits. For instance, rivers with high flows tend to separate into secondary channels and create caves and pools due to scouring processes. These spaces provide different ecological conditions for fishes, including zones with varied water speed, light level, water temperature or vegetation density, which promote diversity within the fish community. On the other hand, this supports that high flows do not necessarily lead to rivers with higher abundance of fishes, which is coherent with findings from other studies that show that fish abundance consistently declined in response to both elevated and reduced flow magnitude (Poff et al., 2010).

Two of the metrics of magnitude that presented the highest correlation and significance with fish metrics were flow in April and spring. Other monthly and seasonal metrics presented lower correlation. These results indicate that the sensitivity of monthly and seasonal metrics to fish metrics highly depend on the geographical scales. A possible explanation for this is that when using hydrological metrics at a national scale, data become much more heterogenous. Thus, it is difficult for monthly and seasonal hydrological metrics to represent common seasonal and monthly patterns that are, at the same time, able to show sensitiveness to biological metrics, unless these patterns are very distinct, as it is the case for flow values in spring (or April in this case). This assumes that in Spain swelling stream flows occur mainly in spring, or more specifically, and according to the studied sample, in April, due to the snowmelt and the increased precipitation associated to this period. From this, it can be concluded that, at a national scale, only very defined patterns flow magnitude are sensitive and useful, where in Spain, and considering the studied sample, it is the flow magnitude in spring, more specifically April. Other monthly flow metrics could be more suitable in studies at smaller scales (e.g. Tejerina-Garro & Mérona, 2010; Carlisle et al., 2017; Fornaroli et al., 2020).

The results suggested that the higher the annual maximum flow, the higher the richness in rivers. At the same time, results also suggested that rivers whose peak flow occur in autumn or winter tend to have a lower richness, whereas rivers whose peak flows occur in spring, tend to have a higher richness. Therefore, high maximum flows occurring in spring are important flow components to ensure high diversity of fishes in the Spanish rivers. This is a clear indicator of the importance of the timing, and not just the magnitude, of the hydrological regime components (Richter et al., 1997). Additionally, the fact that the metric flow in April had a higher correlation with richness metrics than the metric flow in spring, could be used to recommend metrics that are not based on month, but timing of specific flow conditions in the seasonal cycle, as suggested by Yarnell et al., (2020). For instance, that study, developed in California, identified that the start date of the peak flushing flow (autumn pulse flow) was associated with upstream migration of fall-run Chinook Salmon (Peterson et al., 2017). Following this example, a potentially relevant metric for Spain would be the start date of spring flows. Indeed, many studies have shown the importance of spring high flows, together with spring warmer water temperatures, for life-history signals for fish species. More specifically, some cyprinids species, which spend the winter in middle and low courses where the temperature is milder, identify the increasing flows in spring as a cue for spawning migration in the upper courses. Furthermore, spring flows play an important role in larva development (Fornaroli et al., 2020).

The richness of native species was greater in rivers with higher maximum flow magnitude and spring flow magnitude. In contrast, the proportion of alien individuals was greater in rivers with lower flow magnitude. Therefore, it is concluded that high flows in spring enhance the diversity of native species and are associated to low proportions of alien individuals. Indeed, there are studies that show that timing and magnitude reliability of natural high flows likely improves the resistance of native fish communities to alien fish invasions (Fausch et al., 2001). It has also been proven that reservoirs, whose storage usually depletes autumn minimum flow magnitudes and annual high flow magnitudes in spring, are related to the spread of alien species (Vinyoles et al., 2007).

Concerning abundance metrics, the results suggested that fish abundance decreases in rivers with a higher number of annual flow reversals. A natural hydrological regime has regular flow increases followed by progressive flow recessions produced by precipitation events. However, reversals in the streamflow are more common in rivers regulated by reservoirs that need to satisfy either local agricultural demands, which implies diversions and returns of flow, or hydropower generation, which implies intermittent water releases (Carlisle et al., 2017). Fish communities inhabiting this sort of environments need to deal with continuous water level fluctuations (i.e. hydropeaking) and its effects, including changes in water speed and physicochemical conditions. Indeed, the effects of hydroelectric dams on fish communities have been widely documented (e.g. Robinson, 2012; Benejam et al., 2016). For instance, brown trout recruitment is negatively affected by high water velocity and hydropeaking (Dibble et al., 2015). In addition, metrics describing erratic flows has been associated to fish communities impairment both in studies implemented at a national (Carlisle et al., 2017; Praskievicz & Luo, 2020) and local (Belmar et al., 2018) scales. Some studies (Dibble et al., 2015) have computed reversals metrics with hourly flow data series, under the assumption that these records will better characterize the water fluctuations that affect sites downstream from hydroelectric dams during a day. This, of course, will give a more precise quantification of reversals, but hourly data is generally not available in studies at a large scale. Thus, it can be concluded that daily resolution is accurate enough to compute hydrological metrics related to flow reversals that are sensitive to fish metrics. Furthermore, reversals metrics are useful when studying fish-hydrological relationships at a national scale, because they tend to lead to lower fish abundance values.

Abundance metrics were also positively associated with skewness and variability of annual flows, being skewness the highest correlated. Rivers in dryland are characterized by a high range of variation (Walker et al., 1995), as in Spain, and this leads to fish species adapting to this variation. These results are coherent with other studies that have found, at a national scale, that reduced skewness is associated to impairment of fish communities (Carlisle et al., 2017). Consequently, this metric is meaningful when studying fish-flow relationships at a national scale in regions where hydrographs present high variations between maximum and median flows.

Fish abundance aspects also are likely to be influenced by the timing of the sampling. For instance, after the spawning season, higher density of fishes is expected to be found in rivers, with stronger representation of alevin specimens. However, this was unable to be identified in the results since the sampling was conducted only once and during the spawning season, and thus alevin individuals were not present yet. If relationships between fish abundance and hydrological timing components should be studied, interannual samplings would be necessary. A sampling in the early spring and in the late summer would be recommended to evaluate differences in this metric. Another factor that can influence fish abundance is precedent flow condition. For instance, if a severe drought occurred in summer, the alevin and juvenile survivorship decreases, consequently influencing a fish sampling taking place in autumn. For this reason, several studies in the reviewed literature analyze eco-hydrological relationships with varied short antecedent hydrological period (e.g. Carlisle et al., 2017; Belmar et al., 2018), such as metrics computed with the 12 months, 2 years and 5 years precedent hydrological records. Another way to address bias in abundance metrics caused by precedent flow conditions is to inspect hydrographs of the years precedent to the fish sampling. This way, sites affected by singular flow conditions can be discarded, and results better interpreted. This was not done for this study, and thus it is an actual limitation.

Special attention deserves the metric abundance of individuals longer than 150 mm. This metric was positively correlated with skewness metric while negatively correlated with reversals metric. Assuming that fishes whose total length is longer than 150 mm are in an adult stage, this indicates that fish communities barely develop in rivers affected by flow reversals. On the other hand, a fish communities develop in rivers that preserve high flow pulses (i.e. skewed streamflow regimes). This is coherent with other results that suggest that native fishes are not harmfully affected by severe floods (Marchetti & Moyle, 2001). In addition, this reinforces the capacity of streamflow to have an effect on recruitment (Dibble et al., 2015). Recruitment is the transition process from young to adult stages in fishes.

Fish metrics related to trophic interaction did not show any relationships with hydrological metrics, except for the proportion of omnivorous relative to total number of individuals. Even metrics related to invertivorous species, which were promising metrics in this study because they have been documented in the literature as good indicators of hydrological alteration due to their vulnerability to disturbances (e.g. Ferreira et al., 2007) and sensitiveness to hydrological metrics (Belmar et al., 2018), did not show any relationship. According to the obtained results, the proportion of omnivorous individuals increases as flow magnitude increases. This metric has also shown correlation with hydrological metrics in former studies with hydrological impaired sites, assuming that disturbances lead to opportunistic omnivorous diets (e.g. Tejerina-Garro & Mérona, 2010). However, it is difficult to make conclusion for this group of metrics, because there is a lack of information about trophic specialization for some species (4) in the study sample, and some other species are not associated to a single trophic guild (e.g. Oncorhynchus mykiss is defined as both invertivore and piscivore). The ambiguity and lack of consistency of Iberian fish traits, especially those categorical ones such as trophic specialization, is a problem that could lead to low reliability (Cano-Barbacil et al., 2020), as it is the case in this study. In this sense, and until ecological traits are not well defined, it seems more plausible to avoid metrics related to fish trophic specialization in studies at a large scale. Their use is still suitable for studies at a basin level (Belmar et al., 2018), where the number of species is lower, and their ecological traits may be better known.

While the proportion of individuals tolerant to habitat degradation was positively correlated with skewness, the proportion of individuals intolerant to habitat degradation was positively correlated with base flow. These two relationships suggested that rivers with skewed regimes (i.e. high differences between the annual maxima flow and the annual mean flow) is home to fish communities with greater proportion of individuals tolerant to habitat degradation. On the contrary, rivers with low base flows (i.e. minimum flows similar in magnitude to median flows) is home to fish communities with greater proportion of individuals intolerant to habitat degradation. In other words, fishes tolerant to habitat degradation thrive in rivers with maximum flow variability, while fishes intolerant to habitat degradation prefer rivers with minimum flow conditions more constant. No findings have been found in the literature to support this result; thus, it cannot be considered as reliable results from this study. Indeed, fish tolerance metrics have been found problematic in the literature due to the ambiguity of these terms, a lack of agreement in their implications and the absence of information about the tolerance or intolerance features of certain fish species (e.g. Maceda-Veiga et al., 2014).

Fish metrics related to ecological traits (e.g. trophic interaction or tolerance) could have led to better results if the type of fish river, namely cyprinid and salmon rivers, had been considered. Ecological traits can substantially vary across river types. This was not done in this study due to the low size of the sample, and thus, it is a limitation for the relationships between metrics related to ecological traits and streamflow.

Finally, even though this study only considered hydrological conditions as a predictor of fish community characteristics, it is relevant to mention that fishes, as any other biological element, are affected by many other environmental aspects. Some important determinants in fish assemblages identified in this study are water temperature, water flow speed (Praskievicz & Luo, 2020) and water quality (Maceda-Veiga et al., 2014). For this reason, the relationships found in this study were not causal. Complementing research on fish-hydrological relationships with other ecological elements such as water quality is essential to provide improved understanding and encourage as holistic as possible management approaches.

5. Conclusions

In this study, eco-hydrological relationships between fish and long-term flow regimes were investigated. Grounding the eco-hydrological relationships found through the statistical analysis substantially helped to came up with more robust conclusions. The following fish-flow relationships were reached:

- In general, rivers with higher flows tended to have a greater fish diversity than rivers with lower flows. More specifically, high flow in April was concluded to be one of the most consistently dominant metrics of magnitude and timing that could be particularly important for studies examining links between fish assemblages and hydrological regimes at a national scale. It was found that high flows in April could also endorse native species richness in rivers below dams, preventing the settlement of alien species.
- Fish abundance was found to decrease in rivers affected by flow reversals and increase in rivers with variability of high flows. In this sense, it was found that fish

communities likely mature in rivers with flow variability and no affected by reversals.

- In general, metrics related to ecological traits were not explicative at a national scale in Spain, likely due to certain ambiguity in data and lack of information. Their use in large areas whose rivers are poor in fish species and rich in endemicity was not recommended until additional investigations refine this information.

6. References

Acreman, M. C., & Ferguson, A. J. D. (2010). Environmental flows and the European Water Framework Directive: Environmental flows and WFD. *Freshwater Biology*, *55*(1), 32-48. https://doi.org/10.1111/j.1365-2427.2009.02181.x

Acreman, M. C., Overton, I. C., King, J., Wood, P. J., Cowx, I. G., Dunbar, M. J., Kendy, E., & Young, W. J. (2014). The changing role of ecohydrological science in guiding environmental flows. *Hydrological Sciences Journal*, *59*(3-4), 433-450. https://doi.org/10.1080/02626667.2014.886019

Aparicio, E., Carmona-Catot, G., Moyle, P. B., & García-Berthou, E. (2011). Development and evaluation of a fish-based index to assess biological integrity of Mediterranean streams: IBI MEDITERRANEAN STREAMS. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *21*(4), 324-337. https://doi.org/10.1002/aqc.1197

Aparicio, E., Vargas, M. J., Olmo, J. M., & de Sostoa, A. (2000). Decline of Native Freshwater Fishes in a Mediterranean Watershed on the Iberian Peninsula: A Quantitative Assessment. *Environmental Biology of Fishes*, *59*(1), 11-19. https://doi.org/10.1023/A:1007618517557

Arthington, A. H., Bunn, S. E., Poff, N. L., & Naiman, R. J. (2006). The Challenge of Providing Environmental Flow Rules to Sustain River Ecosystems. *Ecological Applications*, *16*(4), 1311-1318.

Bailey, R. C., Norris, R. H., & Reynoldson, T. B. (2004). Bioassessment of Freshwater Ecosystems. En R. C. Bailey, R. H. Norris, & T. B. Reynoldson (Eds.), *Bioassessment of Freshwater Ecosystems: Using the Reference Condition Approach* (pp. 1-15). Springer US. https://doi.org/10.1007/978-1-4419-8885-0_1

Balcombe, S. R., Arthington, A. H., Thoms, M. C., & Wilson, G. G. (2011). Fish assemblage patterns across a gradient of flow regulation in an Australian dryland river system: FLOW REGULATION INFLUENCES FISH ASSEMBLAGE PATTERNS. *River Research and Applications*, *27*(2), 168-183. https://doi.org/10.1002/rra.1345

Belmar, O., Vila-Martínez, N., Ibáñez, C., & Caiola, N. (2018). Linking fish-based biological indicators with hydrological dynamics in a Mediterranean river: Relevance for environmental flow regimes. *Ecological Indicators*, *95*, 492-501. https://doi.org/10.1016/j.ecolind.2018.06.073

Benejam, L., Saura-Mas, S., Bardina, M., Solà, C., Munné, A., & García-Berthou, E. (2016). Ecological impacts of small hydropower plants on headwater stream fish: From individual to community effects. *Ecology of Freshwater Fish*, *25*(2), 295-306. https://doi.org/10.1111/eff.12210

Bonada, N., Dolédec, S., & Statzner, B. (2007). Taxonomic and biological trait differences of stream macroinvertebrate communities between mediterranean and temperate regions: Implications for future climatic scenarios. *Global Change Biology*, *13*(8), 1658-1671. https://doi.org/10.1111/j.1365-2486.2007.01375.x

Brisbane Declaration. (2007). *Environmental flows are essential for freshwater ecosystem health and human well-being*. 3-6.

Bunn, S. E., & Arthington, A. H. (2002). Basic Principles and Ecological Consequences of Altered Flow Regimes for Aquatic Biodiversity. *Environmental Management*, *30*(4), 492-507. https://doi.org/10.1007/s00267-002-2737-0

Cano-Barbacil, C., Radinger, J., & García-Berthou, E. (2020). Reliability analysis of fish traits reveals discrepancies among databases. *Freshwater Biology*, *65*(5), 863-877. https://doi.org/10.1111/fwb.13469

Carlisle, D. M., Grantham, T. E., Eng, K., & Wolock, D. M. (2017). Biological relevance of streamflow metrics: Regional and national perspectives. *Freshwater Science, 36*(4), 927-940. https://doi.org/10.1086/694913

Clavero, M., Blanco-Garrido, F., & Prenda, J. (2004). Fish fauna in Iberian Mediterranean river basins: Biodiversity, introduced species and damming impacts. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *14*(6), 575-585. https://doi.org/10.1002/aqc.636

Colin, N., Porte, C., Fernandes, D., Barata, C., Padrós, F., Carrassón, M., Monroy, M., Cano-Rocabayera, O., de Sostoa, A., Piña, B., & Maceda-Veiga, A. (2016). Ecological relevance of biomarkers in monitoring studies of macro-invertebrates and fish in Mediterranean rivers. *Science of The Total Environment*, *540*, 307-323. https://doi.org/10.1016/j.scitotenv.2015.06.099

Colin, N., Villéger, S., Wilkes, M., de Sostoa, A., & Maceda-Veiga, A. (2018). Functional diversity measures revealed impacts of non-native species and habitat degradation on species-poor freshwater fish assemblages. *Science of The Total Environment*, *625*, 861-871. https://doi.org/10.1016/j.scitotenv.2017.12.316

DGA, D. G. del A., & CEDEX, C. de E. E. de O. P. (2018). *Síntesis de los Planes Hidrológicos Españoles: Segundo ciclo de la DMA (2015–2021).*

Dibble, K. L., Yackulic, C. B., Kennedy, T. A., & Budy, P. (2015). Flow management and fish density regulate salmonid recruitment and adult size in tailwaters across western North America. *Ecological Applications*, *25*(8), 2168-2179. https://doi.org/10.1890/14-2211.1

Doadrio, I., Perea, S., Garzón-Heydt, P., & González, J. L. (2011). *Ictiofauna Continental Española Bases para su seguimiento*.

EPA, U. S. E. P. A. (2011). *Biological assessments: Key Terms and Concepts*.

Fausch, K. D., Karr, J. R., & Yant, P. R. (1984). *Regional Application of an Index of Biotic Integrity Based on Stream Fish Communities*. 18.

Fausch, K. D., Taniguchi, Y., Nakano, S., Grossman, G. D., & Townsend, C. R. (2001). FLOOD DISTURBANCE REGIMES INFLUENCE RAINBOW TROUT INVASION SUCCESS AMONG FIVE HOLARCTIC REGIONS. *Ecological Applications*, *11*(5), 18.

Ferreira, T., Oliveira, J., Caiola, N., De Sostoa, A., Casals, F., Cortes, R., Economou, A., Zogaris, S., Garcia-Jalon, D., Ilhéu, M., Martinez-Capel, F., Pont, D., Rogers, C., & Prenda, J. (2007). Ecological traits of fish assemblages from Mediterranean Europe and their responses to human disturbance. *Fisheries Management and Ecology*, *14*(6), 473-481. https://doi.org/10.1111/j.1365-2400.2007.00584.x

Fornaroli, R., Muñoz-Mas, R., & Martínez-Capel, F. (2020). Fish community responses to antecedent hydrological conditions based on long-term data in Mediterranean river basins (Iberian Peninsula). *Science of The Total Environment*, *728*, 138052. https://doi.org/10.1016/j.scitotenv.2020.138052

Freeman, M. C., Bowen, Z. H., Bovee, K. D., & Irwin, E. R. (2001). FLOW AND HABITAT EFFECTS ON JUVENILE FISH ABUNDANCE IN NATURAL AND ALTERED FLOW REGIMES. *Ecological Applications*, *11*(1), 12.

García de Jalón, D., González del Tánago, M., & Casado, C. (1992). *Ecology of regulated streams in Spain: An overview*. 6.

García-Berthou, E., Almeida, D., Benejam, L., Magellan, K., Bae, F., Casals, F., & Casals, R. (2014). Ecological impact of inland fish introduced to the Iberian Peninsula. *Ecosistemas*, *24*(1), 36-42. https://doi.org/10.7818/ECOS.2015.24-1.07

Gartzia de Bikuña, B., Arrate, J., Martínez, A., Agirre, A., Azpiroz, I., & Manzanos, A. (2017). Development of a fish-based index to assess the ecological status of oceanic-temperate streams in the Northern Iberian Peninsula. *Fundamental and Applied Limnology*, *189*(4), 315-327. https://doi.org/10.1127/fal/2017/0982

Grantham, T. E., Figueroa, R., & Prat, N. (2013). Water management in mediterranean river basins: A comparison of management frameworks, physical impacts, and ecological responses. *Hydrobiologia*, *719*(1), 451-482. https://doi.org/10.1007/s10750-012-1289-4

Hermoso, V., Clavero, M., Blanco-Garrido, F., & Prenda, J. (2009). Assessing freshwater fish sensitivity to different sources of perturbation in a Mediterranean basin. *Ecology of Freshwater Fish*, *18*(2), 269-281. https://doi.org/10.1111/j.1600-0633.2008.00344.x

Hooke, J. M. (2006). Human impacts on fluvial systems in the Mediterranean region. *Geomorphology*, *79*(3-4), 311-335. https://doi.org/10.1016/j.geomorph.2006.06.036

Hughes, R. M., Kaufmann, P. R., Herlihy, A. T., Kincaid, T. M., Reynolds, L., & Larsen, D. P. (1998). A process for developing and evaluating indices of fish assemblage integrity. *Canadian Journal of Fisheries and Aquatic Sciences*, *55*(7), 1618-1631. https://doi.org/10.1139/f98-060

Junk, W., Bayley, P. B., & Sparks, R. E. (1989). The flood pulse concept in river-floodplain systems. *Canadian Journal of Fisheries and Aquatic Sciences*.

Karen, R., & Aldo, V. (2017). *waterData: Retrieval, Analysis, and Anomaly Calculation of Daily Hydrologic Time Series Data* (R package version 1.0.8) [Computer software]. https://CRAN.R-project.org/package=waterData

Karr, J. R. (1981). *Assessment of Biotic Integrity Using Fish Communities*. *6*(6), 8.

Karr, J. R., & Dudley, D. R. (1981). Ecological perspective on water quality goals. *Environmental Management*, *5*(1), 55-68. https://doi.org/10.1007/BF01866609

Karr, J. R., Fausch, K. D., Angermeier, P. L., Yant, P. R., & Schlosser, I. J. (1986). Assessing biological integrity in running waters. A method and its rationale. *Illinois Natural History Survey*.

Kondolf, G. M. (1997). PROFILE: Hungry Water: Effects of Dams and Gravel Mining on River Channels. *Environmental Management*, *21*(4), 533-551. https://doi.org/10.1007/s002679900048

Kondolf, G. M., & Batalla, R. J. (2005). Chapter 11 Hydrological effects of dams and water diversions on rivers of Mediterranean-climate regions: Examples from California. En *Developments in Earth Surface Processes* (Vol. 7, pp. 197-211). Elsevier. https://doi.org/10.1016/S0928-2025(05)80017-3

Logez, M., Pont, D., & Ferreira, M. T. (2010). Do Iberian and European fish faunas exhibit convergent functional structure along environmental gradients? *Journal of the North American Benthological Society*, *29*(4), 1310-1323. https://doi.org/10.1899/09-125.1

Lytle, D. A., & Poff, N. L. (2004). Adaptation to natural flow regimes. *Trends in Ecology & Evolution*, *19*(2), 94-100. https://doi.org/10.1016/j.tree.2003.10.002

Maceda-Veiga, A., Green, A. J., & De Sostoa, A. (2014). Scaled body-mass index shows how habitat quality influences the condition of four fish taxa in north-eastern Spain and provides a novel indicator of ecosystem health. *Freshwater Biology*, *59*(6), 1145-1160. https://doi.org/10.1111/fwb.12336

Marchetti, M. P., & Moyle, P. B. (2001). Effects of flow regime on fish assemblages in a regulated California stream. *Ecological Applications*, *11*(2), 530-539. Scopus. https://doi.org/10.1890/1051-0761(2001)011[0530:EOFROF]2.0.CO;2

Mathews, R., & Richter, B. D. (2007). Application of the Indicators of Hydrologic Alteration Software in Environmental Flow Setting. *Journal of the American Water Resources Association*, *43*(6), 1400-1413.

Menezes, S., Baird, D. J., & Soares, A. M. V. M. (2010). Beyond taxonomy: A review of macroinvertebrate trait-based community descriptors as tools for freshwater biomonitoring: Trait-based community descriptors. *Journal of Applied Ecology*, *47*(4), 711-719. https://doi.org/10.1111/j.1365-2664.2010.01819.x

Mezger, G., González del Tánago, M., & De Stefano, L. (2021). Environmental flows and the mitigation of hydrological alteration downstream from dams: The Spanish case. *Journal of Hydrology*, *598*, 125732. https://doi.org/10.1016/j.jhydrol.2020.125732

MITECO, (Ministerio para la transición ecológica). (2021a). *Inventario nacional de presas y embalses*. https://sig.mapama.gob.es/snczi/

MITECO, (Ministerio para la transición ecológica). (2021b). https://www.miteco.gob.es/es/prensa/ultimas-noticias/la-reserva-hidr%C3%A1ulica-espa%C3%B1ola-se-encuentra-al-523-por-ciento-de-su-capacidad/tcm:30-483552

Moyle, P. B., & Marchetti, M. P. (1999). Applications of Indices of Biotic Integrity to California Streams and Watersheds. En *Assessing the Sustainability and Biological Integrity of Water Resources Using Fish Communities*. CRC Press.

Moyle, P. B., Marchetti, M. P., Baldrige, J., & Taylor, T. L. (1998). *Fish Health and Diversity: Justifying Flows for a California Stream*. *23*(7), 11.

Nick, B. (2019). *hydrostats: Hydrologic Indices for Daily Time Series Data* (R package version 0.2.7) [Computer software]. https://CRAN.R-project.org/package=hydrostats

Olden, J. D., & Poff, N. L. (2003). Redundancy and the choice of hydrologic indices for characterizing streamflow regimes. *River Research and Applications*, *19*(2), 101-121. https://doi.org/10.1002/rra.700

Parrish, J. D., Braun, D. P., & Unnasch, R. S. (2003). Are We Conserving What We Say We Are? Measuring Ecological Integrity within Protected Areas. *BioScience*, *53*(9), 851. https://doi.org/10.1641/0006-3568(2003)053[0851:AWCWWS]2.0.CO;2

Peterson, M. L., Fuller, A. N., & Demko, D. (2017). Environmental Factors Associated with the Upstream Migration of Fall-Run Chinook Salmon in a Regulated River. *North American Journal of Fisheries Management*, *37*(1), 78-93. https://doi.org/10.1080/02755947.2016.1240120

Poff, N. L., Allan, J. D., Bain, M. B., Karr, J. R., Prestegaard, K. L., Richter, B. D., Sparks, R. E., & Stromberg, J. C. (1997). The Natural Flow Regime. *BioScience*, *47*(11), 769-784. https://doi.org/10.2307/1313099

Poff, N. L., Allan, J. D., Palmer, M. A., Hart, D. D., Richter, B. D., Arthington, A. H., Rogers, K. H., Meyer, J. L., & Stanford, J. A. (2003). River Flows and Water Wars: Emerging Science for Environmental Decision Making. *Frontiers in Ecology and the Environment*, *1*(6), 298-306.

Poff, N. L., Richter, B. D., Arthington, A. H., Stuart, E. B., Naiman, R. J., Kendy, E., Acreman, M., Apse, C., Bledsoe, B. P., Freeman, M. C., Henriksen, J., Jacobson, R. B., Kennen, J.

G., Merritt, D. M., O'keeffe, J. H., Olden, J. D., Rogers, K., Tharme, R. E., & Warner, A. T. (2010). The ecological limits of hydrologic alteration (ELOHA): A new framework for developing regional environmental flow standards. *Freshwater Biology*, *55*, 147-170.

Poff, N. L., & Zimmerman, J. K. H. (2010). Ecological responses to altered flow regimes: A literature review to inform the science and management of environmental flows. *Freshwater Biology*, 12.

Pont, D., Hugueny, B., & Rogers, C. (2007). Development of a fish-based index for the assessment of river health in Europe: The European Fish Index. *Fisheries Management and Ecology*, *14*(6), 427-439. https://doi.org/10.1111/j.1365-2400.2007.00577.x

Praskievicz, S., & Luo, C. (2020). Assessment of flow–ecology relationships for environmental flow standards: A synthesis focused on the southeast USA. *Hydrological Sciences Journal*, *65*(4), 571-582. https://doi.org/10.1080/02626667.2020.1714051

Ramos, V., Formigo, N., & Maia, R. (2018). Environmental Flows Under the WFD Implementation. *Water Resources Management*, *32*(15), 5115-5149. https://doi.org/10.1007/s11269-018-2137-8

Raskin, P., Gleick, P., Kirshen, P., Pontius, G., & Kenneth, S. (1997). *Water Futures: Assessment of Long-range Patterns and Problems*. 92.

N.º 1 (2001).

Richter, B., Baumgartner, J. V., Powell, J., & Braun, D. P. (1996). A Method for Assessing Hydrologic Alteration within Ecosystems. *Conservation Biology*, *10*(4), 1163-1174.

Richter, B., Baumgartner, J., Wigington, R., & Braun, D. (1997). How much water does a river need? *Freshwater Biology*, *37*(1), 231-249. https://doi.org/10.1046/j.1365-2427.1997.00153.x

Richter, B. D., Warner, A. T., Meyer, J. L., & Lutz, K. (2006). *A collaborative and adaptive process for developing environmental flow recommendations*. 22.

Robinson, C. T. (2012). Long-term changes in community assembly, resistance, and resilience following experimental floods. *Ecological Applications*, *22*(7), 1949-1961. https://doi.org/10.1890/11-1042.1

Segurado, P., Caiola, N., Pont, D., Oliveira, J. M., Delaigue, O., & Ferreira, M. T. (2014). Comparability of fish-based ecological quality assessments for geographically distinct Iberian regions. *Science of The Total Environment*, *476-477*, 785-794. https://doi.org/10.1016/j.scitotenv.2013.09.004

Segurado, P., Ferreira, M. T., Pinheiro, P., & Santos, J. (2008). *Mediterranean river assessment. Testing the response of guild-based metric.*

Taylor, V., Jewitt, G., & Schulze, R. (Unpublished). *Indicators of Hydrologic Alteration for Assessing Environmental Flows for Highly Variable River*.

Tejerina-Garro, F. L., & Mérona, B. de. (2010). Flow seasonality and fish assemblage in a tropical river, French Guiana, South America. *Neotropical Ichthyology*, *8*(1), 145-154. https://doi.org/10.1590/S1679-62252010005000005

Urbanič, G., & Podgornik, S. (2008). *Testing some European fish-based assessment systems using Slovenian fish data from the Ecoregion Alps*. 13.

Vinyoles, D., Robalo, J. I., de Sostoa, A., Almodóvar, A., Elvira, B., Nicola, G. G., Fernández-Delgado, C., Santos, C. S., Doadrio, I., Sardà-Palomera, F., & Almada, V. C. (2007). *SPREAD OF THE ALIEN BLEAK ALBURNUS ALBURNUS (LINNAEUS, 1758) (ACTINOPTERYGII, CYPRINIDAE) IN THE IBERIAN PENINSULA: THE ROLE OF RESERVOIRS*. 10.

Walker, K. F., Sheldon, F., & Puckridge, J. T. (1995). A perspective on dryland river ecosystems. *Regulated Rivers: Research & Management*, *11*(1), 85-104. https://doi.org/10.1002/rrr.3450110108

Ward, J. V. (1998). Riverine landscapes: Biodiversity patterns, disturbance regimes, and aquatic conservation. *Biological Conservation*, *83*(3), 269-278. https://doi.org/10.1016/S0006-3207(97)00083-9

WCD, (World Commission on Dams). (2000). *Dams and development: A new framework for decision-making.*

WFD, - Water Framework Directive. (2003). *Common implementation strategy for the water framework directive (2000/60/EC).* (Guidance document N.º 7).

World Commission on Dams. (2000). *Dams and development: A new framework for decision-making: The report of the world commission on dams.*

Worrall, T. P., Dunbar, M. J., Extence, C. A., Laizé, C. L. R., Monk, W. A., & Wood, P. J. (2014). The identification of hydrological indices for the characterization of macroinvertebrate community response to flow regime variability. *Hydrological Sciences Journal*, *59*(3-4), 645-658. https://doi.org/10.1080/02626667.2013.825722

Yarnell, S. M., Stein, E. D., Webb, J. A., Grantham, T., Lusardi, R. A., Zimmerman, J., Peek, R. A., Lane, B. A., Howard, J., & Sandoval-Solis, S. (2020). A functional flows approach to selecting ecologically relevant flow metrics for environmental flow applications. *River Research and Applications*, *36*(2), 318-324. https://doi.org/10.1002/rra.3575