Exploring longitudinal trends and recovery gradients in macroinvertebrate communities and biomonitoring tools along regulated rivers

Andrés Mellado-Díaz^{1,2*}, Jorge Rubén Sánchez-González^{1,2}, Simone Guareschi^{3,4},
Fernando Magdaleno⁵, Manuel Toro Velasco¹

(1) Centro de Estudios Hidrográficos (CEDEX). Paseo Bajo de la Virgen del Puerto, 3, 28005, Madrid, Spain.

(2) Present address: Gerencia de Planificación y Gestión Hídrica. TRAGSATEC. C/ Valentín Beato 6, 28037, Madrid, Spain.

(3) Department of Ecology and Hydrology, Regional Campus of International Importance “Campus Mare Nostrum”, University of Murcia, Espinardo Campus, 30100 Murcia, Spain.

(4) Present address: Geography and Environment, Centre for Hydrological and Ecosystem Science, Loughborough University, Loughborough, Leicestershire, LE11 3TU, UK.

(5) Unidad de Apoyo - Dirección General del Agua. Ministerio para la Transición Ecológica. Plaza San Juan de la Cruz, s/n. 28071 Madrid.

(*) Corresponding author: Andrés Mellado-Díaz. Email address: amellado@um.es

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Abstract

Flow regime alteration by dams has been recognized as a major impact factor for aquatic communities. Spain is currently the member state of the EU with the largest number of large reservoirs. With the broad objective of diminishing the ongoing river degradation trend through the management of environmental flows and the use of biomonitoring tools, we investigated the effects of dams on stream macroinvertebrates in several regulated rivers in Spain with contrasting environmental settings. Specifically, we studied longitudinal trends in macroinvertebrate communities to test: i) if currently used biomonitoring tools and multivariate community analyses can detect hydrological impact responses and biological recovery; ii) if an applicable quantification of the recovery gradient, in terms of distance downstream from dams, can be obtained for Iberian fluvial systems; iii) if macroinvertebrate community structure respond different to flow regulation, depending on the contrasting environmental river typologies; and iv) if the type and intensity of hydrological alteration modulates the observed community responses/recovery.

Biotic indices and metrics displayed a decrease in 5 out of 6 systems immediately downstream of infrastructure. Complete recovery could not be clearly detected, but some recovery patterns started at a distance greater than 11km. Multivariate community patterns and biomonitoring metrics showed the most pronounced hydrological alteration impacts and weaker recovery of the downstream macroinvertebrate communities within dammed Mediterranean streams (comparing to other rivers with continental or oceanic climate influence). Finally, both the intensity and type of hydrological alteration (highlighting the alteration of the floods and droughts components) were related to changes in common biomonitoring metrics. Our results could help in recognizing heavily modified water bodies (*sensu* European Water Framework Directive)

downstream of dams or the delineation of fluvial zones or reserves. Furthermore, applied research areas dealing with environmental flows or the bioassessment of hydrological impacts could benefit from our main findings.

Keywords: Ecohydrology, Dams, Longitudinal patterns, Aquatic communities, Bioindicators, Modified rivers

1 Introduction

Multiple stressors and threats affect freshwater biota and the function of lotic ecosystems at local and basin scales (Dudgeon et al., 2006; Strayer and Dudgeon, 2010; Ormerod et al., 2010; Laini et al., 2018; Reid et al., 2018). Hydrological variation caused by river impoundments is a substantial contributor to aquatic habitat alteration and community disturbance. Flow and its variations (i.e. flow regime) have long been recognized as a major structuring force of stream communities (Poff et al., 1997; Poff, 2018) and several studies have demonstrated a relationship between hydrological regime and aquatic-riparian communities worldwide (Pringle et al., 2000; Bunn and Arthington, 2002; Monk et al., 2006; Belmar et al., 2013; Bruno et al., 2014; Solans and Mellado-Díaz, 2015; Rolls et al., 2018; Staentzel et al., 2018; Tonkin et al., 2018). Hydraulic infrastructures such as dams can also have important impacts on sediment fluxes and dynamics. For instance, by sediment trapping, dams deprive downstream reaches of sediments essential for channel form and aquatic habitats (Kondolf et al. 2014). On the other hand, the downstream release of large quantities of fine sediment during specific hydraulic operations (i.e. sediment flushing) can additionally affect stream habitats and communities (Doretto et al. 2019).

Environmental consequences of dams include direct impacts to stream biota, and lotic macroinvertebrate communities are specifically influenced by hydromorphological conditions and flow alterations, both in the upstream and downstream sections (Poff and Zimmerman, 2010; Mendoza-Lera et al. 2012; Guareschi et al., 2014; González et al., 2018; Meißner et al., 2018; Krajenbrink et al., 2019).

Macroinvertebrate community is functionally very important in river ecosystems. They are involved in numerous ecosystem processes such as biomass production, nutrient cycling and resource processing (e.g. Covich et al., 1999) and their communities are currently used as model systems for biomonitoring programmes worldwide (Buss et al., 2015). Moreover, links between macroinvertebrates and flow regime greatly affect river ecosystem functioning (Elosegi and Sabater, 2013; Sabater et al., 2018) and can have consequences even on terrestrial consumers and neighbouring systems (Greenwood and Booker, 2016).

Dams can be considered as discontinuities within the river continuum (Vannote et al., 1980; Ellis and Jones, 2013) and their cumulative impacts on stream ecosystems can be severe (Grill et al., 2015). The effect of dams has been mostly investigated in the immediate vicinity of infrastructure from both the ecological and hydromorphological point of view (e.g. Ligon et al., 1995; Graf, 2006; Magdaleno and Fernández-Yuste, 2011; Lobera et al., 2015). In this context, the serial discontinuity concept (SDC: Ward and Stanford, 1983; Ellis and Jones, 2013) predicts a physical, chemical, and biological recovery towards unregulated conditions as distance downstream from the point of discontinuity increases (Stanford and Ward, 2001). The SDC paradigm, later integrated in a wider conceptual framework by the Riverine Ecosystem Synthesis (Thorp et al., 2006), was proposed and described for North American rivers, with longitudinal trends in aquatic biota downstream of dams mostly investigated along US and Canadian

regulated systems (e.g., Ellis and Jones, 2013, 2016; Holt et al., 2015; Jones and Schmidt, 2018). Specific recovery gradients along large downstream river stretches have not been widely studied or tested thus far in Europe (but see Camargo and Voelz, 1998).

Dams and reservoirs are considered as an increasing global threat to river conservation worldwide (Zarfl et al., 2015). This is particularly important in Europe, with about 7,000 large dams and over 230,000 river barriers registered in 13 EU countries (EEA, 2018; Schiermeier, 2018). In Spain, the EU member state with the largest number of reservoirs (Sabater, 2008; Lehner et al., 2011; Lobera et al., 2015; González del Tánago et al., 2016), the intensive exploitation of rivers dates back to first half of the twentieth century, and more than 1,500 large dams and over 50,000 small dams, weirs and other transversal obstacles are documented (CIREF, 2016).

The impacts of dam regulation on aquatic communities are integrally associated with the storage capacity of the reservoirs, their water release capabilities and the purpose (e.g. water supply, energy supply, flood alleviation). These impacts can also depend on the environmental, climate, hydrological and land-use settings (e.g. Belmar et al., 2019). Thus, Mediterranean climate streams can be more prone to hydrological alteration than temperate rivers, because water demand is greater and runoff is not in line with demand (Kondolf and Batalla, 2005) and because unpredictable precipitation and limited water availability have resulted in extensive water infrastructure development (Grantham et al., 2010). There is a significant lack of knowledge on the different ecological responses to damming and altered flow regimes. This lack of research is more accentuated in Mediterranean climate rivers and streams compared to temperate ones (Olden et al., 2014; Gilliespie et al., 2015). Thus, it is critical to better understand and model these

responses, trying to mitigate the impacts on stream biota with the broad objective of diminishing these ongoing river degradation trends.

To fill these gaps, we investigated the effects of dams on macroinvertebrate communities in several regulated lotic systems in Spain. Specifically, we studied longitudinal trends in macroinvertebrate communities (using diversity indices and other biomonitoring metrics as well as multivariate community structure) to test: i) if currently used biomonitoring tools and multivariate community analyses can detect hydrological impact and potential recovery (i.e. a return to pre-dam conditions, measured in terms of biotic indices values, or in terms of community structure, as the returning to an original position in a multidimensional space); ii) if an applicable quantification of the recovery gradient, in terms of distance downstream from dams, can be obtained for Iberian fluvial systems; iii) if macroinvertebrate community structure respond differently to flow regulation depending on the environmental setting, with Mediterranean rivers being more affected than temperate systems; and iv) if the type and intensity of hydrological alteration modulate the impact on communities and their downstream responses.

In this context, we expected to observe a certain dam-impact gradient. More precisely, we expected a maximum dam impact (i.e., the lowest diversity and biomonitoring metrics values, and the largest distance from reference sites in community structure using multivariate ordination space) at the sites located immediately downstream of dams; whereas at the lower-most sites (downstream of the dam), we expected higher diversity and biomonitoring metrics values, and lower distance from reference sites in community structure using multivariate ordination space.

This distance would represent the longitudinal space necessary for a variable (or a multivariate space position) to effectively recover to pre-impoundment conditions. The

assessment and estimation of these potential recovery trends and distances can contribute to the science of regulated rivers ecology and provide relevant information for bioassessment methods that rely on the classification of ecological status of stream segments, setting the basis for the longitudinal delimitation of heavily modified water bodies (HMWB, *sensu* WFD 2000/60/CE) downstream of reservoirs. Moreover, it would allow for hierarchical allocation of rehabilitation or restoration measures to improve the ecological status of affected reaches, by more accurately identifying and mitigating impaired ecological processes.

2 Methods

2.1 Study area

Due to the high number of reservoirs or barriers in Spain, it was very difficult to find regulated rivers with sections downstream of dams longer than 40-60 km that were not affected by other pressures, mostly in Mediterranean areas, regarded as one of the most heavily impacted regions by humans (Hooke, 2006). After intense screening, six regulated stream reaches (30-40 km) along different forested mountain ranges in peninsular Spain (Southern Europe, Figure 1; Table 1) were selected.

Stream reaches with relatively consistent morphological, geological and land use characteristics and specifically without major anthropogenic alterations were considered to avoid factors that could confound the interpretation of results. Climatic and environmental differences between regulated river catchments occurred (Table 1), with two typical Mediterranean climate rivers located in southern Spain (Guadalquivir and Cabriel), two rivers in Central Spain with continental climate influence (Najerilla and Jarama) and two rivers in northern Spain with oceanic climate influence (Porma and Avia). Furthermore, when possible, reference conditions were obtained from the

following unregulated streams (see methodological details below): Tea river (as reference for Avia), Iregua (as reference for Najerilla), Torío (as reference for Porma) and Sorbe (as reference for Jarama) (Figure 1). Reservoir characteristics and uses, as well as environmental settings of the studied systems are summarized in Table 1.

2.2 Sampling sites and study design

Based on a literature review on recovery distances downstream of dams (summarized in Ellis and Jones, 2013), at least 3 sites located up to 40 km downstream of the dam, separated by ca. 10 km, were sampled in each regulated system. At least one reference site was also located upstream the dams (at least 5 km away from the tail end of the reservoir). Furthermore, two reference sites were also sampled in the nearest comparable unregulated stream when available (in four out of the six rivers). Similar study designs have been applied in other ecohydrological case studies (e.g. Braatne et al., 2008; Olden and Naiman, 2010; Jones, 2010; Ellis and Jones, 2013).

Three seasonal sampling campaigns during spring, summer and autumn were performed during 2009 and a total of 92 macroinvertebrate samples were analysed for the study (details for each system in Table 1). At each site (100 meters reach), a multi-habitat sample was taken using a kick-net (500 μ m mesh) and consisted of 20 “kicks” distributed among different substrates or microhabitats, in proportion to their surface cover area. Each “kick” covered 0.125 m² (20 kicks covering 2.5 m²). Macroinvertebrates were identified to family level (the required taxonomic level for biomonitoring purposes in Spain) following Tachet et al. (2010), except for Hydracarina, Ostracoda and Oligochaeta, which were identified as such.

2.3 Community variables and biotic indices

Ecological integrity and macroinvertebrate communities were evaluated using the following metrics: the Iberian Biomonitoring Working Party (IBMWP) and the Iberian Average Score per Taxon (IASPT) biotic indices, richness (total number of families), EPT richness (number of families in Ephemeroptera, Plecoptera and Trichoptera) and Shannon diversity index H' . The IBMWP index is the official invertebrate biomonitoring index currently used in Spain and is one of the most widely used in Iberian rivers (Alba-Tercedor et al., 2002; Munné and Prat, 2009; MAGRAMA, 2015). Its relative, the IASPT index, is the IBMWP value divided by the number of scoring families. It is routinely utilised within multi-metric approaches and used to guide biomonitoring practices across Spain (e.g., Sánchez-Montoya et al., 2010; Guareschi et al., 2017). Richness, EPT richness and Shannon diversity index H' are common biomonitoring metrics widely applied in bioassessment worldwide (Magurran, 2004; Birk et al., 2012; Doretto et al., 2018).

Additionally, as a measure of the specific flow-related impacts on aquatic biota, a modification of the Lotic-invertebrate Index for Flow Evaluation (LIFE) (Extence et al., 1999) was also used. The LIFE index evaluates the flow preference of the macroinvertebrate community by assigning macroinvertebrate families to 6 flow preferences (I-VI): from those of torrential waters (class I, $v > 100$ cm/s, e.g. Blephariceridae) to those typical of lentic systems (class V, $v = 0$ cm/s, e.g. Chaoboridae) or even to drought resistant families typically inhabiting temporary systems (class VI, e.g. Triopsidae). In this study, each family was assigned to its flow preference class (following Extence et al., 1999) and values were weighted by their relative abundance in a sample. When adding these weighted values, a continuous indicator was obtained, bounded from 1 to 6 (maximum flow to preference for temporary lentic systems) which we called LIFE_{mod}.

2.4 Environmental variables and hydrological alteration

Fluvial habitat integrity was evaluated by means of the Fluvial Habitat Index (IHF, Pardo et al., 2002) while the quality of the riparian forest and hydromorphological alteration was evaluated by the QBR index (*Qualitat del Bosc de Ribera*, in English: Riparian Forest Quality, Munné et al., 2003). Both have been widely used in riparian ecosystem or in-stream habitat assessment in Spain. Moreover, the QBR represents the official tool for hydromorphological assessment following Spanish legislation (MAGRAMA, 2015).

In all sampling dates and sites, data of basic physicochemical parameters were also measured in situ using standard procedures or instrumentation: Multi-Parameter Water Quality Sonde YSI 6600 for T^a, pH, conductivity and dissolved oxygen; titration with phenolphthalein for alkalinity (Rice et al., 2012); filtration through glass fibre filters (Millipore 47mm Ø 0.45µm) for total suspended solids (AENOR, 1996); and Photometer LASA[®] 20 methods for main nutrients (NH₄-N, NO₂-N, NO₃-N). Substrate size was visually estimated for each “kick” as one of 9 size classes, from fines (0) to rock bed (9) and averaged for the whole stream segment. Site averaged environmental data are summarized in Suppl. Mat. SM_1.

To assess the general hydrological alteration of the studied reaches, a number of indices of hydrological alteration (IHAs) were calculated following the methodology of Richter et al. (1996), by means of two approaches depending on data availability: a) by comparing reference flow data periods before and after the building of the dams (Porma, Najerilla and Jarama), or b) by comparing un-impacted flow reference gauges located upstream the dams with their altered counterparts downstream (Guadalquivir and Cabriel). In the Avia river, as no data were available before the dam construction or

from upstream gauges, an unimpacted gauge in the nearby Tea river was used as a reference. Downstream gauges were selected as near as possible to the dam. Information on gauging stations and flow series used in the analyses is available as Suppl. Mat. SM_2. The IAHRIS 2.2 software was used to quantify flow patterns' shifts (Martínez and Fernández, 2010a; 2010b). The IAHRIS software calculates a total of 22 indices dealing with the magnitude, variability, seasonality and duration of the three main components of the flow regime: habitual values, floods and droughts. To make interpretation easier, we focused on the "global alteration indices" (gIHAs), that are combinations of the indices calculated for each component. All gIHA indicators vary in the range 0-1 (with 0 representing maximum alteration, and 1 representing the absence of alteration), and five different "hydrological status" levels are established: "very good", "good", "moderate", "deficient" and "bad" (see Martínez and Fernández 2010a and 2010b for details). In parallel, as an indicator of the potential hydrological alteration caused by each reservoir, the IR index (Impoundment-Runoff index, Batalla et al., 2004) was calculated. The IR index is the total capacity of a reservoir divided by the mean annual runoff entering the reservoir.

2.5 Data analyses

Sites downstream of dams were classified in 5 potential impact classes based on distance from the infrastructure responsible to interrupt the fluvial continuum: class 5 (0-4 km distance, maximum impact), class 4 (4-10km), class 3 (10-20 km), class 2 (20-30 km) and class 1 (>30 km). Class 0 was used for reference sites upstream of dams or in free-flowing comparable streams.

To understand the spatial-temporal variability of environmental variables, a principal component analysis (PCA) was carried out using standardized data. Different river

systems, as well as the different seasons, were displayed in the PCA plot to visualize the main trends.

Non-metric multidimensional scaling (NMDS) analyses were performed to identify the major distribution patterns in macroinvertebrate communities. Prior to analysis, macroinvertebrate community abundances were $\log_{10}(x+1)$ transformed. Bray–Curtis distance was used as the dissimilarity measure, and stress was used as a goodness of fit surrogate. Linear fittings, using the R-vegan function *envfit*, were performed between environmental variables and the NMDS ordination axes to identify the main environmental factors driving the composition of the benthic communities. The significance of the fitted vectors was assessed using a permutation procedure (9999 permutations).

To test for differences among impact classes and sampling campaigns in each system, a nonparametric permutational multivariate analysis of variance (PERMANOVA) was carried out using impact class (i.e. the position in the longitudinal gradient) and season, as well as the interaction of both, as factors.

To unravel the effects of damming and possible recovery trends, all the community metrics and biomonitoring tools considered were analysed within a linear mixed models' framework to avoid pseudoreplication issues (LMM; Zuur et al., 2009, Bates et al., 2019). Sites were grouped as follows: classes 1 and 2 into L group (large distance, >20 km from the hydraulic infrastructure), classes 3-4 into M group (medium distance, 4-20 km), while class 0 represented the C group (upstream or reference sites) and class 5 the S group (short distance, 0-4 km). By taking into account the characteristics of the experimental design, the number of data and statistical assumptions, “river” was included as a random effect, while “group” was considered as fixed. We used an analysis of deviance to perform a likelihood ratio test comparing the full model to a null

model to determine whether “group” had a significant effect on all the biological metrics. Pairwise contrasts of modelled treatment means were performed using the *emmeans* function for multiple comparisons (Lenth et al. 2019), a method recently applied in similar comparisons with terrestrial invertebrates (Myers et al. 2019). Homoscedasticity and normality of residuals (graphical inspection and Shapiro–Wilk test) were also checked.

Correlation analysis (Spearman) was used to test for possible relationships between changes in community metrics (calculated as the deviation from the mean reference values of class 0 sites of the same river system) and indices of hydrological alteration (IR and gIHAs). All statistical analyses were performed using the statistical computing software R (R 3.4.0 version; R Core Team, 2018) with the packages: *vegan* (Oksanen et al., 2018), *ade4* (Dray and Dufour, 2007), *lme4* (Bates et al., 2019) and *emmeans* (Lenth et al., 2019),

3 Results

3.1 Environmental variables and hydrological alteration

Regarding general spatiotemporal variability in the environmental variables, the first two axes of the PCA (Figure 2) explained 43% of the variance. Axis 1 was positively correlated to conductivity, alkalinity and pH (and to a lesser degree to suspended solids and fine substrates) and can be interpreted as a lithology axis (Figure 2). It explained 26% of variance and separated the Guadalquivir and Cabriel basins (calcareous), from the others (more siliceous). Axis 2 (17% variance) was negatively correlated with temperature (water and air) and positively with dissolved oxygen, clearly separating the three sampling seasons, as well as subtly signalling the more thermal Mediterranean character of the Cabriel and Guadalquivir rivers, in opposition to the continental-

oceanic influences of the other basins (Figure 2). Site averaged environmental data are shown in Suppl. Mat. SM_1.

Hydrological alteration indices (Table 2) showed strong alteration in the “droughts” component in 3 of the studied rivers (Cabriel, Avia and Jarama rivers), where hydrological status was classified as bad. Hydrological status was classified as “bad” for “habitual values” in the Guadalquivir River and as “deficient” for the Cabriel River. The “floods” component indicated a “moderate” hydrological status in 4 cases (Cabriel, Guadalquivir, Avia and Porma rivers). In general, the Porma and Najerilla rivers showed the lowest hydrological alteration values, while the Guadalquivir and Cabriel rivers did not achieve a “good” hydrological status for any component.

3.2 Environmental variables shaping macroinvertebrate communities

Multivariate analyses (NMDS plus *envfit*) showed that variables related to climate (water temperature) and lithology (conductivity, alkalinity and pH), together with the concentration of nitrate, were most strongly related to variations in the aquatic macroinvertebrate communities (Figure 3). The Guadalquivir and Cabriel basins (calcareous, Mediterranean climate, richer in nutrients) were separated from the others (siliceous, continental or oceanic-influenced, nutrient-poor).

To circumvent the strong influence asserted by these climatic and geological differences between catchments, we also analysed the multivariate patterns for each system separately (results showed in Suppl. Mat. SM_3). At most sites, the increasing dam impact gradient was found in gradients of decreasing IHF index, although in the Guadalquivir an inverted trend was observed, with lower IHF values in reference sites. Nitrate was also an influential variable in the Guadalquivir and Cabriel rivers, while nitrite and conductivity were more important in the northern streams Porma and Avia.

Temperature was not a main factor explaining longitudinal changes in macroinvertebrate communities in any of the studied streams, being just significant in the Najerilla, but clearly not discriminating among dam impact classes but among different seasons (see PERMANOVA results below). Substrate size was the only significant factor for Jarama river communities (although not related to the longitudinal impact gradient), while regulated Mediterranean streams located in southern watersheds (the Cabriel and Guadalquivir rivers) showed pronounced community changes along the downstream gradient, linked to IHF, conductivity and nitrate variations (Suppl. Mat. SM_3). On the other hand, more northern temperate streams showed much shorter trajectories (smaller community changes) correlated to changes in IHF and nitrite concentrations. The Najerilla and Porma rivers were characterized by somewhat cyclical recovery trends whereas the Avia and Jarama, showed more erratic patterns (Figure 3; Suppl. Mat. SM_3).

PERMANOVA results stressed the relevance of position along the regulation gradient in Mediterranean systems (Guadalquivir and Cabriel, with significant p-values and the higher R^2 values, Table 3) while seasonality was especially important for the Najerilla-Iregua and Jarama-Sorbe systems as well as for the Guadalquivir river (p-values <0.001). In contrast, the influence of regulation on macroinvertebrate communities was not significant for the northern oceanic climate rivers Avia and Porma.

3.3 Variation in macroinvertebrate community metrics

Longitudinal variations in community metrics and biotic indices differed between catchments depending on climatic and environmental settings, as well as the type and intensity of hydrological alterations caused by reservoir management. (Figure 4).

The IBMWP index generally decreased below dams with some slight trend changes in class 3 (10-20 km) or class 2 (20-30 km) reaches (except the Cabriel, with an increase in the most downstream class 1, and the Avia river, with a slight increase in the first section and decreasing afterwards). A fairly similar pattern was observed for total taxa richness. The EPT richness metric always decreased below dams, showing signs of recovery in class 3 or 2 reaches, especially for the Jarama, Porma and Najerilla rivers, and in class 1 for the Cabriel, but continued decreasing downstream in the Guadalquivir river. Similarly, the Shannon biodiversity index generally decreased, except for the first kilometres of the Avia (followed by a constant decrease after this) and Jarama rivers, with some recovery tendency being shown once reaching classes 4 or 3 (4-20 km downstream). The LIFEmod index generally increased below dams, with recovery occurring by class 4 or 3 reaches, with the exception of the Jarama river, which showed a slight decrease in the first section and continuing decreasing afterwards. The IASPT index generally decreased without any recovery trend along most of the entire study area.

Significant results were obtained when comparing full models (group as fixed effect) to null models (just intercept) stressing the relevance of “group” (p-value<0.05 for each variables). Considering the global behaviour of the metrics and pairwise comparisons among groups, 5 of them showed some significant differences between the reference sites of class 0 (group C) and the other groups (except for the LIFEmod index), indicating a damming effect on freshwater communities (Table 4). Significant results were obtained when comparing group C with groups S, M and L for IBMWP, IASPT and EPT richness, while Shannon index presented significant difference just between group C and group S. Results are qualitative similar, with some more marked effects, when considering a subset of 86 samples (93% of the total samples) based on the

distance from the water release instead of the distance from the hydraulic infrastructure (Suppl. Mat. SM_4ab).

The correlation between biological metrics deviation (from mean class 0 upstream-reference values) and the different hydrological alteration metrics (IR and gIHAs) are shown in Table 5. The IR index was significantly correlated with changes in IBMWP and IASPT. Concerning the global gIHAs, the habitual values component (gIHAh) was correlated with IBMWP changes only, the flood component (gIHAfl) was correlated to changes in richness, EPT richness, IBMWP and IASPT, while the drought component (gIHAdr) was significantly correlated to EPT richness and IBMWP. Focusing on the different community metrics, the IBMWP index was correlated with all the hydrological alteration indices, while Shannon H' and the LIFEmod index were not correlated to any of them (Table 5). Plots for these correlations are shown in Suppl. Mat. SM_5.

4. Discussion

4.1. Longitudinal community gradients

Impacts of flow regulation by dams (for energy production, water supply, flood control and other uses) are increasingly recognized as a global threat to freshwater ecosystems and their services to human communities (Poff et al., 2007; Grill et al., 2015; Zarfl et al., 2015; Moran et al., 2018). Understanding the effects of hydrological alterations on riverine communities and ecosystems, as well as proposing possible conservation and mitigation strategies, is therefore of vital importance for the preservation of ecological integrity and to achieve a sustainable water management.

Our study represents a novel investigation regarding the potential recovery of aquatic communities along longitudinal gradients below dams in different environmental settings along the Iberian Peninsula. The study was able to identify some biological

patterns along the first 30-40 km downstream of hydraulic infrastructure (aims i and ii). However, an extension of the studied longitudinal stretches seems worthy of further research. Indeed, other environmental variables may require a more substantial longitudinal study area. For example, recovery of temperature, one of the most affected variables below large reservoirs with deep hypolimnetic releases, has been estimated to take between 40 and 930 km (see Olden and Naiman, 2010 and references therein). Water chemistry, on the contrary, usually exhibits rapid attenuation in the first kilometers below dams (Ellis and Jones, 2013), depending on the release type and reservoir management. In the studied reaches a variety of community responses, depending on the stream ecotype and the type and intensity of hydrological alteration, was observed (aims iii and iv). The Cabriel and Guadalquivir rivers, strongly regulated and with inverted seasonal patterns (maximum flows in summer, associated with irrigation needs due to the intensive agricultural uses in these catchments), showed strong community variations along the longitudinal gradient. However, while the Guadalquivir seemed most affected by longitudinal changes in community structure, in the Cabriel river, a more cyclical trend trajectory was observed, which we interpret as a moderate recovery in macroinvertebrate communities. As tributaries can influence longitudinal recovery trends (Rice et al., 2008; Jones and Schmidt, 2018), the lack of important tributaries in the Guadalquivir river may be exerting some influence on the observed trends. Unlike these two systems, rivers located in areas with continental-oceanic influence showed much shorter longitudinal trajectories of community change. The Najerilla and Porma rivers were characterized by short cyclical trajectories, with a more probable recovery of the communities, while patterns of change in the Jarama or Avia were more erratic. PERMANOVA analysis of community change supported these results, with lower R^2 and non-significant differences in communities along the

downstream distance gradient in the northern oceanic climate rivers. These contrasting patterns were strongly related to our estimates of hydrological alteration, with the Guadalquivir and Cabriel rivers presenting the highest IR values and not reaching a good hydrological status for any of the gIHA indices. The correlations found between the gIHA indices and the biomonitoring metrics along the downstream impact gradient add strength to our results and confirms our hypothesis that the type and intensity of hydrological alteration affect the observed impact and recovery trends of macroinvertebrate communities.

Fluvial habitat alteration (measured in terms of IHF) downstream of dams appears to be the major disturbance for macroinvertebrate community composition in most of our studied systems. IHF reflects habitat heterogeneity, which is frequently stressed as one of the main drivers of stream community structure and diversity patterns (e.g., Mellado-Díaz et al., 2008; Martinez et al., 2013; Astorga et al., 2014). The impact of flow regime alterations by dams on in-stream physical habitat features and indices has been previously demonstrated at different habitat scales, from whole stream reaches to microhabitats (Kondolf, 1997; Bunn and Arthington, 2002; Wiatkowski and Tomczyk, 2018). The QBR index did not detect, in general, the impacts of the dams on riparian vegetation in downstream reaches, with the exception of the Cabriel River. Given that plant encroachment downstream of dams is a known general trend (Power et al. 1996; García de Jalón et al., 2017) and that vegetation cover can overshadow hydromorphological structure and functioning of riparian areas in this index, the QBR index offered limited value in distinguishing longitudinal trends. Nutrients differentially affected macroinvertebrate communities at our study sites: while nitrates (agricultural sources) primarily affected southern Mediterranean rivers, nitrites (possibly from livestock sources) had some influence in the more northern river systems. Nevertheless,

variability in nitrite concentrations was too low to draw any conclusion about this trend.
(see data in Suppl. Mat. SM_1).

4.2. Biomonitoring tool responses

Overall, a declining trend in our indices and metrics occurred directly downstream of dams as hypothesized. This response was more evident in the Cabriel, Jarama, Najerilla and Porma rivers, whereas the Avia and Guadalquivir rivers often showed an inverted response, with metrics increasing in the first kilometres downstream of dams and decreasing afterwards. The most plausible reason of this trend is that, in these two systems, the release of water from the dam occurs downstream of the first sampling site, which is therefore not affected by peak discharges. The stable flow conditions appeared to promote well-developed aquatic and riparian plant communities and a relatively diverse macroinvertebrate community.

Specifically, IBMWP, EPT richness, IASPT and richness indices displayed an immediate decrease below the dam in 5 out of 6 systems, while Shannon H' decreased in 4 fluvial systems highlighting their potential to detect downstream impacts in regulated rivers (aim i). Martinez et al. (2013) found similar results in Northern Spain, with flow alteration negatively affecting density, richness and diversity of macroinvertebrates in reaches just below dams (distances <1 km). Trends consistent with our hypotheses were also observed for the LIFEmod index, increasing immediately below dams in 5 out of 6 systems (indicating a “lentification” of communities, *sensu* Sabater, 2008) and partially recovering in the downstream gradient (at classes 3-4). Other studies have shown similar results elsewhere (Ellis and Jones, 2013; Meißner et al., 2018), with richness metrics decreasing downstream of dams. In contrast, Krajenbrink et al. (2019) recently found the opposite (increases below dams) in some

UK streams for EPT richness and taxa richness. This could be related to differences in study design (e.g. presence/absence of other impact sources) or hydrological management downstream of UK dams. Nevertheless, other metrics in the same study, such as the WHPT (an invertebrate-based index replacing the BMWP in UK river bioassessments, UKTAG, 2014), the percentage of abundance of EPT (but not the EPT richness) and the LIFE index did show similar trends (decrease in indices values; community identification) as those observed in this study.

Estimation of recovery distances can be useful for longitudinally delimitating heavily modified water bodies (HMWB) in river segments downstream of reservoirs. Our community-based approach to delimitate HMWB downstream of dams can complement other purely hydrological classifications, like the one proposed by Fernández et al. (2012) for Spanish rivers. However, in the context of the SDC concept predictions (and aims i and ii) it seems difficult to quantify a clear and univocal recovery distance applicable for all the Iberian fluvial systems studied. For instance, in the case of IBMWP, IASPT and EPT richness recovery was not observed even at class 4 (4-10 km downstream), suggesting that the effect of the impoundments was not remediated over this distance. Moreover, global results of the pairwise comparisons by groups (C, S, M, L) did not show total recovery at any group distance downstream of dams for the same metrics. However, some qualitative trend changes appeared to begin, in some cases (e.g. IBMWP in 4 systems), by impact class 3 (11-20 km), suggesting a minimum distance of more than 11 km was required in our study area. Only the Shannon index presented significant differences just between reference sites and sites located closest to the infrastructure (group S) indicating a quick recover of this metric after 4 km. Interestingly, Holt et al. (2015), studying the Chattahoochee River (Georgia, USA), found that macroinvertebrate assemblages did not recover from regulation impacts even

65 km downstream of dams. Finally, IBMWP values were always near to or even above reference conditions for every stream typology reflecting, in general, a very good ecological status (e.g. mean IBMWP value of each group > 200) along studied reaches. These high diversity and biotic indices values may represent a strongly resilient community that could, at least partially, affect results (e.g. by rapid re-colonization from upstream or downstream sites). Nevertheless, the selected study reaches avoided other main anthropogenic impacts.

4.3 Management implications and future research

We tested widely used taxonomic and monitoring tools to provide easy and ready-to use information for water managers, environmental authorities, consultancies and researchers that consider similar tools and metrics on a frequent basis. In fact, applied research areas like restoration ecology, biomonitoring, habitat suitability modelling and water resources management could benefit from our approach, experimental design and findings. However, as some responses were type or even system-specific, supplementary research (e.g., different basins, conditions, larger sections and other complementary metrics) would help validate and improve our results. A univocal quantification of recovery distance downstream hydraulic infrastructure remains an open challenge in regulated rivers and ecohydrology not only in Southern Europe (see Holt et al., 2015) and further studies are thus still crucial. A functional diversity focus could also be useful (e.g. with functional metrics and indices, e.g. Ruhi et al., 2018; Laini et al., 2019) to complement and improve our findings and test potential concordances or mismatches among the studied variables and tools. Using indicators of hydrological alteration, our study highlighted the importance of the floods and droughts in shaping the response of macroinvertebrate communities in our study area. These

results stress the importance of extreme values when seeking to restore environmental flows. In this context, it would be of great interest for future studies to test the relationship between the partial IHA indices on stream community changes, focussing on flow components that represent the main hydrological alterations in each case. Here, the length and quality of the flow time series will be critical, since their influence can be decisive in identifying the biological responses to hydrological changes. Finally, in our study, Mediterranean type streams showed the strongest dam-related hydrological alteration and impact and lower levels of downstream community recovery. This highlights the vulnerability of these ecosystems to flow regime alterations (Clavero et al., 2010; Hermoso and Clavero, 2011), as flow alteration is superimposed on natural water scarcity (which is expected to increase with climate change) and increased levels of anthropogenic pressure.

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575 **Tables**

576 **Table 1**

577 List and description of studied reaches and reservoirs (reservoir use codes: HE: hydroelectric; I: irrigation; WS: water supply; FC: flood control).

Regulated river	Reference river	Reservoir	Drainage area (km ²)	Reservoir volume (hm ³)	Average annual runoff (hm ³)	Reservoir main uses	Elevation range (m.a.s.l.)	No. of tributaries ≥ 3rd order below the dam	No. of samples in unregulated / regulated sections	Watershed main land uses (% agriculture / % natural)	Climate; average annual rainfall (mm)	Main lithology
Guadalquivir	Guadalquivir upstream	Tranco	550	498	177	WS, I, HE	684-467	0	4 / 10	<i>Pinus</i> forest; olive groves (37% / 63%)	Mediterranean-Humid 739	Limestones, dolomites and marls
Cabriel	Cabriel upstream	Contreras	3331	852	590	WS, HE, I	912-470	3	4 / 12	<i>Pinus</i> forest; shrublands; Non-irrigated arable land. (17% / 83%)	Mediterranean; 400	Calcarenites, marls and limestones
Jarama	Jarama upstream and Sorbe	Vado	382	56	170	WS, I	1270-722	4	6 / 7	Shrublands; <i>Pinus</i> forest; <i>Quercus</i> forest; crops (6% / 94%)	Continental-Mediterranean; 456	Shales, cuartzites
Najerilla	Najerilla upstream and Iregua	Mansilla	290	68	169	I, WS, HE	1195-642	3	9 / 9	Shrublands; <i>Quercus</i> forest; <i>Pinus</i> forest; <i>Fagus</i> forest (2% / 98%)	Continental-Oceanic; 793	Sandstones, conglomerates, shales and limestones)

Avia	Avia upstream and Tea	Albarellos	214	91	410	HE, FC	466-106	2	9 / 8	<i>Pinus</i> forest; shrublands; Complex cultivation patterns. (14% / 85%)	Continental- Oceanic; 1400	Schists, granites
Porma	Porma upstream and Torío	Porma	253	318	311	WS, I, HE	1140-870	3	7 / 7	Shrublands; <i>Quercus</i> forest; <i>Pinus</i> forest; pastures (14% / 84%)	Continental- Oceanic; 1229	Sandstones, conglomerates, shales and limestones)

578

Table 2

Global indicators of hydrological alteration (gIHAs) downstream of dams in each of the studied river reaches. Three main components of the hydrological regime (values, floods and droughts) are considered. Index values and hydrological status classes are shown.

River	gIHA _h (habitual values)		gIHA _f (floods)		gIHA _{dr} (droughts)	
Guadalquivir	0.03	Bad	0.30	Moderate	0.22	Moderate
Cabriel	0.19	Moderate	0.18	Moderate	0.01	Bad
Jarama	0.09	Deficient	0.47	Good	0.00	Bad
Najerilla	0.36	Moderate	0.66	Very good	0.43	Good
Avia	0.44	Good	0.25	Moderate	0.00	Bad
Porma	0.20	Moderate	0.19	Moderate	0.53	Good

Table 3. Results of the permutational analysis of variance (PERMANOVA) of invertebrate communities. The position in the dam impact gradient (*Dam Impact*) and seasonality (*Season*) were considered as factors, as well as their interaction (*Dam Impact:Season*). Significant *p* values in bold.

River system	Effect	df	SS	MeanS	F	R ²	<i>p</i> (>F)
Guadalquivir	<i>Dam Impact</i>	4	1.17	0.29	6.12	0.32	0.0039
	<i>Season</i>	2	1.58	0.79	16.54	0.44	0.0003
	<i>Dam Impact:Season</i>	5	0.76	0.15	3.20	0.21	0.0229
Cabriel	<i>Dam Impact</i>	4	1.45	0.36	2.09	0.45	0.0390
	<i>Season</i>	2	0.52	0.26	1.51	0.16	0.1700
	<i>Dam Impact:Season</i>	6	0.76	0.13	0.73	0.23	0.8440
Jarama-Sorbe	<i>Dam Impact</i>	3	0.73	0.24	2.03	0.29	0.0427
	<i>Season</i>	2	0.98	0.49	4.06	0.38	0.0002
	<i>Dam Impact:Season</i>	3	0.36	0.12	0.99	0.14	0.5140
Najerilla-Iregua	<i>Dam Impact</i>	3	0.58	0.19	2.26	0.19	0.0211
	<i>Season</i>	2	1.32	0.66	7.70	0.44	0.0001
	<i>Dam Impact:Season</i>	6	0.59	0.10	1.15	0.20	0.3239
Avia-Tea	<i>Dam Impact</i>	3	0.58	0.19	1.28	0.20	0.2360
	<i>Season</i>	2	0.90	0.45	2.95	0.31	0.0070
	<i>Dam Impact:Season</i>	5	0.51	0.10	0.67	0.18	0.8710
Porma-Torío	<i>Dam Impact</i>	3	0.31	0.10	0.62	0.16	0.8740
	<i>Season</i>	2	0.55	0.27	1.64	0.28	0.1100
	<i>Dam Impact:Season</i>	3	0.30	0.10	0.59	0.15	0.8780

Table 4. Results of paired comparisons among groups (C, S, M, L) after linear mixed-effect models. All samples from all the river systems were considered in the analyses. Group C (references or upstream sites, n=39); Group S (short distance, class 5, 0-4 km); Group M (medium distance, classes 3 and 4, 4-20 km); Group L (large distance, classes 1 and 2, >20 km from the hydraulic infrastructure). Significant *p* values in bold.

IASPT					
Contrast	estimate	SE	df	t.ratio	<i>p.value</i>
C - S	0.34	0.11	84.1	3.20	0.0104
C - M	0.49	0.10	84.3	4.74	0.0001
C - L	0.56	0.11	84.1	5.14	0.0001
S - M	0.15	0.11	79.2	1.43	0.4879
S - L	0.22	0.11	79.5	1.94	0.221
M - L	0.06	0.11	79.8	0.58	0.9377
IBMWP					
Contrast	estimate	SE	df	t.ratio	<i>p.value</i>
C - S	38.93	12.9	85.4	3.02	0.0172
C - M	54.11	12.6	85.6	4.31	0.0003
C - L	38.26	13.1	85.5	2.92	0.0228
S - M	15.18	13.0	79.2	1.17	0.6465
S - L	-0.67	13.6	79.7	-0.05	1
M - L	-15.84	13.4	80.1	-1.18	0.6414
Shannon Index					
Contrast	estimate	SE	df	t.ratio	<i>p.value</i>
C - S	0.32	0.11	86.7	2.88	0.0256
C - M	0.15	0.11	85.8	1.35	0.5362
C - L	0.15	0.11	85.7	1.33	0.5458
S - M	-0.17	0.12	80.3	-1.48	0.4551
S - L	-0.17	0.12	82.5	-1.37	0.519
M - L	0.01	0.12	84.5	0.04	1
EPT richness					
Contrast	estimate	SE	df	t.ratio	<i>p.value</i>
C - S	3.83	0.88	83.80	4.37	0.0002
C - M	4.27	0.85	83.90	5.00	0.0001
C - L	3.22	0.89	83.80	3.61	0.0028
S - M	0.44	0.88	79.10	0.50	0.959
S - L	-0.61	0.92	79.40	-0.67	0.9088
M - L	-1.05	0.91	79.70	-1.16	0.6567
Richness					

Contrast	estimate	SE	df	t.ratio	<i>p.value</i>
C - S	4.47	2.13	87.20	2.10	0.1612
C - M	6.09	2.07	87.40	2.94	0.0215
C - L	3.51	2.16	87.30	1.62	0.3715
S - M	1.62	2.16	79.40	0.75	0.8759
S - L	-0.96	2.26	80.10	-0.42	0.9742
M - L	-2.58	2.24	80.90	-1.15	0.6581
LIFEMod					
Contrast	estimate	SE	df	t.ratio	<i>p.value</i>
C - S	-0.20	0.09	86.60	-2.20	0.1315
C - M	0.01	0.09	86.80	0.11	0.9996
C - L	0.18	0.09	86.70	2.02	0.1897
S - M	0.20	0.09	79.30	2.28	0.112
S - L	0.38	0.09	79.90	4.02	0.0007
M - L	0.17	0.09	80.60	1.86	0.2539

Table 5. Spearman correlations (ρ) between biological metrics deviation from mean upstream reference values and hydrological alteration indices (IR and gIHAs). Significant ρ and p-values in bold.

	IR	<i>p-value</i>	gIHA _h	<i>p-value</i>	gIHA _n	<i>p-value</i>	gIHA _{dr}	<i>p-value</i>
Richness	0.25	0.074	-0.24	0.085	-0.4080	0.002	-0.25	0.0768
EPT richness	0.05	0.707	-0.03	0.845	-0.6259	0.000	-0.54	0.0000
Shannon H'	-0.03	0.811	-0.11	0.452	-0.2535	0.067	-0.24	0.0838
IBMWP	0.28	0.040	-0.27	0.049	-0.52	0.000	-0.33	0.0150
IASPT	0.36	0.008	-0.22	0.111	-0.56	0.000	-0.25	0.0692
LIFEmod	-0.09	0.526	-0.08	0.587	0.05	0.710	-0.20	0.1592

Figure captions

Figure 1. Map of peninsular Spain showing the location of the regulated (reservoirs in brackets) and reference catchments: 1. Avia (Albarelllos) and Tea; 2. Porma (Porma) and Torío; 3. Najerilla (Mansilla) and Iregua; 4. Jarama (El Vado) and Sorbe; 5. Cabriel (Contreras); 6. Guadalquivir (Tranco de Beas).

Figure 2. Ordination of samples along the first two axes of PCA analyses based on environmental variables (43% of explained variance). Different catchments and seasons are depicted with inertia ellipses for emphasis, with labels in the centres of gravity. The correlation circle is shown at bottom right: the direction and length of the arrows show the strength of correlation between variables and axes.

Figure 3.

NMDS ordination plot of macroinvertebrate samples. Longitudinal trajectories are plotted by arrows for each stream, from reference-upstream sites (small open circles), then those immediately downstream of dams (biggest circles) and more downstream sites. Environmental parameters fitted by *envfit* ($p < 0.05$) are superimposed as vectors. Stress is shown as a goodness of fit measure.

Figure 4.

Longitudinal trends in community metrics and biomonitoring indices along the dam-impact gradient (i.e. downstream distance), from class 0 (reference sites) to class 5 (max. impact) and then to classes 4 to 1 (see methods).

APPENDIX

Suppl. Mat. SM_1

Environmental variables measured for each sampling site (mean $\pm$ sd). The position on the longitudinal regulation impact gradient (grad_reg) is included, as well as the fluvial habitat and riparian forest quality indices. IHF: Fluvial habitat index value (0-100); QBR: Riparian Forest Quality index (0-100); Tw: water temperature (°C); Ta: air temperature (°C); Oxy: dissolved oxygen (mg/l); cond: conductivity (μ S/cm); alk: alkalinity (meq/l); NH₄: ammonium (mg/l); NO₂: nitrite (mg/l); NO₃: nitrate (mg/l); SS: suspended solids (mg/l); substrate: substrate size class (ordinal, from fines (0) to rock bed (9)). The IHF index was calculated once (SD value not available).

River system	sampling site	grad_reg	QBR	IHF	Tw	Ta	Oxy	pH	cond	alk	NH ₄	NO ₂	NO ₃	SS	substrate
Avia/Tea	Avia01	0	93.33 ± 7.64	68	10.43 ± 2.78	15.53 ± 2.90	10.34 ± 0.38	6.10 ± 0.10	30.20 ± 4.30	0.20 ± 0.17	0.02 ± 0.01	0.01 ± 0.00	0.55 ± 0.13	2.44 ± 3.24	4.57 ± 0.71
Avia/Tea	Tea01	0	90.00 ± 5.00	80	11.52 ± 3.51	16.33 ± 6.01	10.71 ± 1.06	5.72 ± 0.40	21.80 ± 1.71	0.17 ± 0.06	0.03 ± 0.02	0.01 ± 0.00	0.35 ± 0.03	0.58 ± 0.34	4.49 ± 0.66
Avia/Tea	Tea02	0	93.33 ± 5.77	68	12.73 ± 2.79	17.20 ± 1.49	10.54 ± 0.95	5.51 ± 0.41	31.93 ± 2.61	0.20 ± 0.10	0.04 ± 0.03	0.01 ± 0.00	0.32 ± 0.02	0.64 ± 0.36	4.58 ± 0.73
Avia/Tea	Avia02	5	87.67 ± 7.51	70	12.42 ± 4.91	17.57 ± 3.71	10.33 ± 1.25	6.55 ± 0.06	44.33 ± 13.43	0.25 ± 0.17	0.04 ± 0.03	0.01 ± 0.00	0.47 ± 0.04	2.56 ± 2.09	4.47 ± 0.80
Avia/Tea	Avia03	4	100.00 ± 0.00	84	13.38 ± 4.35	17.20 ± 1.70	10.31 ± 1.97	6.23 ± 0.11	44.90 ± 10.04	0.30 ± 0.14	0.02 ± 0.02	0.01 ± 0.01	0.61 ± 0.00	0.96 ± 0.55	4.40 ± 0.85
Avia/Tea	Avia04	3	88.33 ± 2.89	52	14.36 ± 5.45	16.60 ± 5.23	10.19 ± 1.14	6.40 ± 0.51	45.93 ± 17.53	0.30 ± 0.10	0.05 ± 0.03	0.01 ± 0.00	0.54 ± 0.11	1.43 ± 0.60	4.38 ± 0.97
Cabriel	Cabriel01	0	70.00 ± 21.21	71	13.19 ± 8.78	18.60 ± 11.17	11.63 ± 0.09	8.25 ± 0.01	651.50 ± 51.62	4.05 ± 0.49	0.02 ± 0.02	0.01 ± 0.00	0.27 ± 0.26	2.00 ± 0.53	4.51 ± 1.29
Cabriel	Cabriel02	0	80.00 ± 0.00	67	12.51 ± 9.57	16.10 ± 12.87	11.90 ± 0.42	8.12 ± 0.09	652.00 ± 21.21	3.90 ± 0.14	0.02 ± 0.02	0.01 ± 0.00	0.40 ± 0.04	2.32 ± 0.76	4.13 ± 0.08
Cabriel	Cabriel03	5	50.00 ± 15.00	66	11.60 ± 6.09	15.67 ± 13.90	11.62 ± 0.49	8.23 ± 0.14	962.67 ± 147.51	3.72 ± 0.29	0.02 ± 0.01	0.01 ± 0.00	0.25 ± 0.21	3.91 ± 5.00	5.10 ± 1.25
Cabriel	Cabriel04	4	95.00 ± 5.00	63	14.07 ± 9.36	16.87 ± 11.31	11.57 ± 0.41	7.96 ± 0.42	909.33 ± 19.30	3.37 ± 0.40	0.03 ± 0.01	0.01 ± 0.00	0.55 ± 0.57	1.44 ± 1.22	4.99 ± 0.62
Cabriel	Cabriel05	2	95.00 ± 5.00	79	18.06 ± 2.07	16.37 ± 12.62	8.04 ± 1.15	7.45 ± 0.07	982.00 ± 100.35	3.90 ± 0.26	0.03 ± 0.02	0.01 ± 0.00	1.01 ± 0.63	1.19 ± 0.56	3.81 ± 0.51
Cabriel	Cabriel06	1	90.00 ± 7.07	68	15.05 ± 0.35	9.35 ± 1.06	9.4 ± 0.00	8.08 ± 0.04	595.00 ± 661.85	4.00 ± 0.28	0.01 ± 0.00	0.01 ± 0.00	1.26 ± 0.11	2.01 ± 1.07	6.00 ± 0.00
Jarama/Sorbe	Jara01	0	65.00 ± 0.00	61	8.65 ± 4.56	16.40 ± 8.20	11.09 ± 1.12	6.50 ± 0.23	27.00 ± 4.24	0.20 ± 0.00	0.04 ± 0.03	0.01 ± 0.00	0.11 ± 0.12	2.00 ± 1.48	4.90 ± 0.14
Jarama/Sorbe	Sorbe01	0	82.50 ± 3.54	73	13.43 ± 11.41	14.70 ± 5.23	8.96 ± 0.76	5.79 ± 1.36	23.00 ± 0.00	0.18 ± 0.04	0.02 ± 0.01	0.01 ± 0.00	0.09 ± 0.08	0.94 ± 0.90	4.99 ± 0.19
Jarama/Sorbe	Sorbe02	0	100.00 ± 0.00	80	14.43 ± 10.47	16.60 ± 6.51	8.36 ± 0.13	7.33 ± 0.79	93.00 ± 9.90	0.95 ± 0.07	0.03 ± 0.01	0.01 ± 0.00	0.09 ± 0.02	1.55 ± 0.89	5.23 ± 0.06
Jarama/Sorbe	Jara02	5	77.67 ± 2.52	75	8.72 ± 3.85	11.57 ± 6.41	9.38 ± 1.25	6.45 ± 0.75	34.57 ± 6.17	0.30 ± 0.10	0.02 ± 0.02	0.01 ± 0.00	0.12 ± 0.01	1.69 ± 1.06	4.79 ± 0.70
Jarama/Sorbe	Jara03	4	90.00 ± 7.07	58	11.69 ± 2.26	13.95 ± 2.47	10.60 ± 1.05	7.66 ± 0.26	119.00 ± 24.04	1.20 ± 0.28	0.02 ± 0.02	0.01 ± 0.00	0.22 ± 0.04	1.04 ± 0.76	5.50 ± 0.08
Jarama/Sorbe	Jara04	2	77.50 ± 3.54	41	11.79 ± 1.05	14.35 ± 0.49	10.09 ± 0.62	7.82 ± 0.21	398.50 ± 12.02	2.45 ± 0.07	0.04 ± 0.04	0.01 ± 0.01	0.19 ± 0.14	0.80 ± 0.27	5.39 ± 0.16
Najerilla/Iregua	Najerilla01	0	81.67 ± 2.89	71	9.68 ± 6.62	14.83 ± 11.65	10.45 ± 1.89	7.96 ± 0.54	257.33 ± 72.47	2.07 ± 0.35	0.04 ± 0.03	0.01 ± 0.00	0.10± 0.06	1.99 ± 1.23	4.59 ± 0.66
Najerilla/Iregua	Iregua01	0	68.33 ± 7.64	66	10.00 ± 8.99	14.17 ± 7.82	10.13 ± 2.52	7.44 ± 0.38	50.73 ± 14.63	0.43 ± 0.21	0.03 ± 0.02	0.00 ± 0.00	0.09 ± 0.00	1.13 ± 0.68	4.89 ± 0.33
Najerilla/Iregua	Iregua02	0	90.00 ± 0.00	78	7.48 ± 4.10	12.97 ± 13.3	11.49 ± 0.93	7.02 ± 1.13	88.00 ± 9.54	0.57 ± 0.15	0.02 ± 0.01	0.00 ± 0.01	0.09 ± 0.06	1.68 ± 0.54	4.25 ± 0.44
Najerilla/Iregua	Najerilla02	5	90.00 ± 0.00	52	9.90 ± 3.78	15.03 ± 7.48	11.28 ± 1.24	7.01 ± 1.44	164.90 ± 17.41	1.40 ± 0.20	0.05 ± 0.05	0.01 ± 0.00	0.20 ± 0.07	1.93 ± 1.32	4.97 ± 0.84
Najerilla/Iregua	Najerilla03	3	91.67 ± 5.77	62	9.72 ± 2.33	13.40 ± 9.65	10.73 ± 0.70	7.66 ± 0.84	187.13 ± 31.08	1.47 ± 0.45	0.02 ± 0.01	0.01 ± 0.01	0.16 ± 0.10	1.69 ± 0.95	4.56 ± 0.64
Najerilla/Iregua	Najerilla04	2	90.00 ± 5.00	79	11.18 ± 5.21	14.00 ± 8.37	11.01 ± 0.77	7.85 ± 0.72	200.73 ± 46.39	1.57 ± 0.45	0.02 ± 0.01	0.01 ± 0.00	0.17 ± 0.08	1.45 ± 0.16	3.98 ± 0.34
Porma/Torio	Porma01	0	62.50 ± 17.68	73	13.97 ± 6.70	10.90 ± 0.28	9.78 ± 0.78	7.44 ± 0.71	168.50 ± 57.28	1.40 ± 0.14	0.05 ± 0.01	0.01 ± 0.00	0.17 ± 0.10	2.19 ± 1.98	4.90 ± 0.35
Porma/Torio	Torio01	0	82.50 ± 3.54	68	10.87 ± 4.24	12.60 ± 6.65	9.04 ± 1.59	7.69 ± 0.05	162.50 ± 45.96	2.20 ± 0.00	0.04 ± 0.00	0.01 ± 0.00	0.11 ± 0.02	1.13 ± 0.64	5.36 ± 0.24
Porma/Torio	Torio02	0	85.00 ± 21.21	83	12.12 ± 4.87	14.40 ± 4.10	8.07 ± 0.09	8.17 ± 0.20	182.50 ± 36.06	2.43 ± 0.04	0.05 ± 0.00	0.01 ± 0.01	0.09 ± 0.00	0.88 ± 0.68	5.17 ± 0.04
Porma/Torio	Porma02	5	75.00 ± 7.07	48	5.71 ± 2.27	12.15 ± 0.92	10.54 ± 1.49	7.69 ± 0.53	146.40 ± 6.22	1.30 ± 0.14	0.03 ± 0.01	0.01 ± 0.00	0.24 ± 0.09	0.84 ± 0.77	3.71 ± 0.00
Porma/Torio	Porma03	3	57.50 ± 3.54	61	9.17 ± 4.48	12.80 ± 0.28	10.60 ± 2.95	8.06 ± 0.64	155.60 ± 40.45	1.65 ± 0.21	0.05 ± 0.02	0.01 ± 0.00	0.14 ± 0.07	2.84 ± 1.12	3.79 ± 0.00
Porma/Torio	Porma04	1	78.33 ± 16.07	60	9.58 ± 1.49	16.47 ± 10.45	10.32 ± 2.30	7.85 ± 0.67	162.67 ± 37.87	1.77 ± 0.25	0.05 ± 0.02	0.01 ± 0.00	0.18 ± 0.07	1.61 ± 0.47	3.96 ± 0.32
Guadalquivir	Tranco01	0	100 ± 0.00	63	13.46 ± 9.31	14.80 ± 14.28	10.82 ± 1.51	8.20 ± 0.05	384.00 ± 15.56	4.35 ± 0.21	0.03 ± 0.02	0.01 ± 0.00	0.23 ± 0.08	1.49 ± 0.42	4.49 ± 1.10
Guadalquivir	Tranco02	0	50.00 ± 14.14	47	13.52 ± 8.45	17.20 ± 12.02	10.37 ± 2.37	7.94 ± 0.10	427.00 ± 21.21	4.40 ± 0.14	0.03 ± 0.02	0.01 ± 0.00	0.16 ± 0.13	4.57 ± 4.48	3.78 ± 0.47
Guadalquivir	Tranco03	5	98.33 ± 2.89	66	12.18 ± 0.73	13.97 ± 7.42	9.13 ± 0.52	7.78 ± 0.28	419.67 ± 36.83	4.13 ± 0.12	0.02 ± 0.02	0.01 ± 0.00	0.48 ± 0.25	1.88 ± 0.93	4.08 ± 0.61
Guadalquivir	Tranco04	4	92.50 ± 3.54	76	10.00 ± 0.28	10.05 ± 4.60	11.52 ± 0.00	8.30 ± 0.00	490.50 ± 37.48	5.00 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	1.09 ± 0.37	2.52 ± 2.67	3.51 ± 1.23
Guadalquivir	Tranco05	3	82.50 ± 17.68	64	9.97 ± 0.10	5.45 ± 3.89	10.08 ± 0.04	8.22 ± 0.23	581.00 ± 113.14	4.95 ± 0.07	0.01 ± 0.00	0.01 ± 0.00	2.30 ± 1.27	2.91 ± 2.60	3.42 ± 1.18
Guadalquivir	Tranco06	2	77.67 ± 2.52	65	10.39 ± 1.01	16.13 ± 12.26	11.26 ± 0.54	8.34 ± 0.02	505.67 ± 94.3	4.60 ± 0.53	0.02 ± 0.03	0.01 ± 0.00	1.99 ± 1.32	3.74 ± 2.21	3.31 ± 0.40

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Suppl. Mat. SM_2

Flow series used in the analyses, with indication of the code and location of the gauging station, and of the name and initial year of operation of the regulating reservoirs.

River	Pre-regulated years	Post-regulated years	Code of gauging station	Name of reservoir / first year of operation	Location of gauging station
Porma	1943-1966	1968-2006	2011	Porma (1968)	0.6 km D/S the dam wall
Najerilla	1942-1960	1961-2011	9034	Mansilla (1960)	0.05 km D/S the dam wall
Jarama	1911-1949	1959-2011	3050	El Vado (1949)	0.03 km D/S the dam wall
Guadalquivir	1985-2011	-	5134	Tranco de Beas (1944)	18.1 km U/S the reservoir tail
	-	1945-2011	5001		4.3 km D/S the dam wall
Cabriel	1915-1940	-	8050	Contreras (1973)	1.8 km U/S the reservoir tail
	-	1969-2011	8130		2.4 km D/S the dam wall
Tea	1970-2011	-	1645	-	-
Avia	-	1973-2011	1637	Albarelos (1973)	4.7 km D/S the dam wall

Suppl. Mat. SM_3

NMDS ordination plots for the different regulated and reference streams. Samples are classified along a dam impact gradient (maximum= 5 to 0=reference sites) and inertia ellipses are plotted for each impact class. Significant environmental parameters (fitted by *envfit*, $p < 0.05$) are superimposed in each plot. Stress is shown as a goodness of fit measure.

Suppl. Mat. SM_4

Boxplot results of each metric response among groups (C, S, M, L, see Table 4). a) in blue considering the entire dataset (92 data); b) with a subset of 86 data considering distance from water release.

Suppl. Mat. SM_5

Linear regression plots between biological metric deviations (from mean upstream-reference values) and the hydrological alteration indices. IR: Impoundment-Runoff index, gIHAh: global index of hydrological alteration for habitual values; gIHAfl: global index of hydrological alteration for floods; gIHAdr: global index of hydrological alteration for droughts. Significant correlations (Spearman) are marked with (*).

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