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**Multi-biologic group analysis for an ecosystem response to longitudinal river  
regulation gradients**

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**ABSTRACT:**

This work assesses the effects of river regulation on the diversity of different instream and riparian biological communities along a relieve gradient of disturbance in regulated rivers. Two case studies in Portugal were used, with different river regulation typology (downstream of run-of-river and reservoir dams), where regulated and free-flowing river stretches were surveyed for riparian vegetation, macrophytes, bryophytes, macroalgae, diatoms and macroinvertebrates. The assessment of the regulation effects on biological communities was approached by both biological and functional diversity analysis. Results of this investigation endorse river regulation as a major factor differentiating fluvial biological communities through an artificial environmental filtering that governs species assemblages by accentuating species traits related to river regulation tolerance. Communities' response to regulation gradient seem to be similar and insensitive to river regulation typology. Biological communities respond to this regulation gradient with different sensibilities and rates of response, with riparian vegetation and macroinvertebrates being the most responsive to river regulation and its gradient. Richness appears to be the best indicator for general fluvial ecological quality facing river regulation. Nevertheless, there are high correlations between the biological and functional diversity indices of different biological groups, which denotes biological connections indicative of a cascade of effects leading to an indirect influence of river regulation even on non-responsive facets of communities' biological and functional diversities. These results highlight the necessary holistic perspective of the fluvial system when assessing the effects of river regulation and the proposal of restoration measures.

## 1. Introduction

River damming is one of the most aggressive processes in freshwater ecosystems (Allan and Castillo, 2007). The effect of manipulating river flows extends beyond the modification of flow regimes and entails substantial environmental changes that affect nature conservation (Hellowell, 1988). Accordingly, river regulation is one of the greatest contributors to the degradation of freshwater ecosystems (EEA, 2018) and threats to endangered species (Bunn and Arthington, 2002).

It is well known that the fluvial ecosystem responds directly to the river's flow regime (Poff et al., 1997; Poff et al., 2010) and reacts to river regulation with biological and functional amendments mirroring river ecosystem health (Bunn and Arthington, 2002; Norris and Thoms, 1999; Poff and Zimmerman, 2010). Nevertheless, scientists are still unable to precisely determine the relationships between biota and modified flow regimes, lacking experimental research regarding this and particularly concerning the reaction of the ecosystems to restoration (Bunn and Arthington, 2002; Dudgeon, 2020; Poff and Zimmerman, 2010).

Past decades have witnessed rapid advances in biodiversity-based methods to measure ecosystem health (C'Brien et al., 2016; Yu et al., 2017). These methods, which were initially dedicated to community taxonomic composition, are now increasingly focused on species biological traits. In this way, such methods can relate indirectly to the functional roles of species in ecosystems and provide a mechanistic understanding of the impacts of anthropogenic stressors (Verberk et al., 2013). This becomes a plausible necessary paradigm shift to understand how biological elements respond to climate change or anthropic disturbances (Cernansky, 2017). A functional trait is a measurable attribute associated with the fitness and performance of an organism (Garnier et al.,

2016; McGill et al., 2006; Pistón et al., 2019; Violle et al., 2007), and functional diversity is often the most ecologically relevant form of information (Leps et al., 2006). Furthermore, an ecosystem's properties depend greatly on the distribution and abundance of these functional characteristics of organisms, determined by the effects of climate or disturbance regimes, both natural and anthropogenic (Díaz et al., 2007; Hooper et al., 2005; O'Hare et al., 2016). Accordingly, trait-based ecology and modeling have become promising tools in community ecology research to solve pressing ecological problems related to changing environmental conditions (e.g., Laughlin et al., 2012; McGill et al., 2006; Webb et al., 2010) and provide greater explanatory power than species-centered approaches for explaining ecosystem functioning (Cadotte et al., 2011). Furthermore, functional diversity can provide useful measures for disentangling multiple interacting stressors (Baattrup-Pedersen et al., 2016) or finding ecological indicators, particularly regarding land use and management changes (Lozanovska et al., 2018; Posenberg et al., 2008). Traits can also be used as essential biodiversity variables; i.e., ecological health indicators that allow the assessment of global biodiversity change (Feio and Dolédec, 2012; Kissling et al., 2018) regardless of their taxonomic identities (Grime, 2006; Moretti et al., 2017; Villéger et al., 2017). Accordingly, trait-based approaches allow for a reduction in analytical complexity by shifting from a focus on the diversity of species to a diversity of function (Dray and Dufour, 2007). Moreover, trait analysis enables the association of climate change and human-caused pressures with biodiversity and ecosystem functions (Allan et al., 2015; Díaz et al., 2011; Feio et al., 2015; Lavorel and Grigulis, 2012; Suchara, 2019). Functionally based approaches have been applied with diverse purposes and in diverse communities in Mediterranean freshwater systems. Examples include the research of Vieira et al. (2012) and that of Feio and Dolédec (2012), on the relationships

of bryophytes and invertebrates with ecological gradients, as well as the work of Aguiar et al. (2018) on the effects of river regulation and land use on riverine plants.

However, determining how biodiversity dynamics, ecosystem processes and abiotic factors interact remains a challenge (Loreau et al., 2001; Lu et al., 2015). In addition, the current ecosystem health assessment paradigm has a strong bias towards fish and macroinvertebrates (O'Brien et al., 2016). Nevertheless, in the last two decades the implementation of the EU-Water Framework Directive (WFD), as well as other worldwide legislative tools, has boosted the number of studies on macrophytes and diatoms (Aguiar et al., 2014; Almeida et al., 2014). However, species interactions and fragmentation in freshwater systems tend to be focused on trophic webs, and our knowledge of the network changes and evolutionary dynamics require further research efforts (Hagen et al., 2012).

In the end, despite the existence of certain pioneering works simultaneously studying different aquatic communities and their relationships (e.g., Feio et al., 2017; Hering et al., 2006; Segurado et al., 2012; Tonkin et al., 2020; Turunen et al., 2019), the scientific research on this topic is still scarce, particularly the relationships between communities and processes, as well as multiple connections (Feio et al., 2017; Hagen et al., 2012; Larsen et al., 2012).

The present work has the objective of assessing the effects of river regulation on the diversity of different instream and riparian communities along the fluvial longitudinal gradient. Particularly, this work aims to evaluate how diverse biological communities (riparian vegetation, aquatic macrophytes, bryophytes, macroalgae, diatoms and macroinvertebrates) react to relaxing a gradient of disturbance in regulated rivers. It is hypothesized that these communities can respond with a noticeable ecological quality improvement along this gradient or, on the other hand, be indifferent to it. Furthermore,

this community response can also be similar or opposite between biological groups. Accordingly, this study encompasses different biological groups and their relationships in Mediterranean rivers, and advances a scientific development regarding the previously mentioned shortcomings of this topic in river ecology.

## 2. Methods

### 2.1 *Field sampling*

The sampling methodology outline was built with two main purposes: to compare the effects of different flow regulation types on the biological communities and to assess the river regulation intensity gradients along the lengths of rivers. To do so, two different case studies were considered, namely, the Lima and Alva Rivers in Portugal, Southwestern Europe. Both rivers are influenced by a Temperate-Mediterranean climate with hot and dry summers alternating with mild and wet winters. The mean annual rainfall in the study areas ranges from approximately 760 to 1550 mm.year<sup>-1</sup> (Agência Portuguesa do Ambiente, 1974a), with mean daily temperature ranging from 10 to 16.7°C (Agência Portuguesa do Ambiente, 1974b). The Lima River is regulated by the Touvedo and Alto Lindoso dams, whose main purposes are hydropower production. Particularly, the Touvedo dam is a run-of-river dam that modulates the turbinated flows from the upstream Alto Lindoso dam and hence provides an additional service of flood protection. Touvedo dam has very little storage capacity, thus being highly dependent on the prevailing flow rate. The low-head powerhouse is located next to the dam and the turbinated waters are released right after the impoundment. Consequently, the entire river course downstream of Touvedo dam is under the effect of hydropeaking. On the other hand, Fronhas dam is a conventional impoundment dam with the same principal

purposes of hydropower production and flood protection. However, in this case, the water is diverted through a pipeline to a power plant located on a reservoir of a different watershed. Accordingly, no water is released downstream of this dam except a reduced stable ecological flow (Appendix A – Table A.1).

Field sampling took place during June and July 2019. In each case study, field sampling was performed at several sampling sites in free-flowing and regulated river stretches along the river longitudinal dimension of the study river. More precisely, in the Lima case study, 13 sampling sites (L1 to L13) were considered as regulated sites and placed downstream of the Touvedo dam. Because upstream of this dam the flow regime is still regulated by the Alto Lindoso dam, the 4 free-flowing sampling sites considered (L14 to L17) were placed in free-flowing tributaries with similar river characteristics. For the Alva case study, 11 sampling sites (A1 to A11) were placed in the river downstream of the Fronhas dam, and 3 (A12 to A14) upstream of the reservoir (Figure 1).

At every sampling site, riparian vegetation (trees, shrubs and lianas), macrophyte vegetation (herbaceous aquatic and riparian species), bryophytes (mosses and liverworts), macroalgae, diatoms and macroinvertebrates were surveyed. Riparian woody species and macrophytes were mostly identified in the field, but approximately 40 vascular plant specimens were collected for later identification in the João Carvalho e Vasconcellos Herbarium (LISI). Bryophyte, macroalgae, diatom and macroinvertebrate samples were collected for species identification and abundance recording in the laboratory. Bryophytes were collected from each sampling site and dried in paper bags for later identification in the laboratory. Collected specimens were identified and deposited as vouchers in Porto Herbarium (PO). The same sampling procedure was adopted for macroalgae, but these were preserved with 10% buffered formalin in jars. Macroalgae were identified to the genus level. Diatoms were collected



from three 1 dm<sup>2</sup> stones of available mesohabitats, scrubbed with a soft brush and preserved with 10% formalin in 60-ml glass bottles. Diatoms were identified to the species level and for each sample a minimum of 400 valves (the cell wall is composed of 2 siliceous valves) were counted (relative abundance). Macroinvertebrate samples were collected with a hand-net (500- $\mu$ m mesh size, 0.25 $\times$ 0.25 m opening) by kick sampling, covering 3 $\times$ 1 m of available mesohabitats (e.g., pool, run and riffle) in each stream reach. Samples were preserved with 10% formalin until further macroinvertebrate sorting, identification and counting. Macroinvertebrates were identified to the highest possible taxonomic level of resolution (i.e., genus and species), except for Diptera (identified to family, subfamily or tribe level) and Oligochaeta (family).

Along with biological data, environmental variables were also recorded at each sampling site, particularly regarding geomorphology and water quality (see Appendix A – Table A.2).

## 2.2 Data analysis

After species identification, three data matrices were built, namely, an environmental matrix (R matrix) with the environmental characterizations of the sampling sites, a taxa matrix (L matrix) with information on species cover/abundance, and a trait matrix (Q matrix) characterizing species traits. Data were saved in data frames where lines stood for species or sampling sites, and columns for abundances, traits or environmental variables, accordingly to the corresponding matrix. Trait information was obtained for each species from databases, using dummy coding for categorical variables and aggregating quantitative variables in mean values that ignore intraspecific variation for each trait. The rationale of this approach is based on the concept of interspecific trait

variation being much greater than intraspecific variation. This fact is particularly used in studies linking community assemblies with trait-by-environment relationships (Gelfand et al., 2019). Traits selected included organisms' biological characteristics that were expected to directly respond to flow regulation changes. Traits were attributed to taxa according to available biological trait databases described by Aguiar et al. (2013), Hill et al. (2004), Klotz et al. (2004) and Willby et al. (2000) for riparian vegetation and macrophytes, Hill et al. (2006) and Hill et al. (2007) for bryophytes, Bellinger and Sigeo (2010) for macroalgae, Cunningham and McMinn (2004) Marra et al. (2016), Rimet and Bouchez (2012), and Rimet et al. (2010) for diatoms, and Tachet (2010) for macroinvertebrates.

A total of 49 traits (10 for riparian vegetation, 9 for macrophytes, 3 for bryophytes, 4 for macroalgae, 13 for diatoms and 10 for macroinvertebrates), considered to be related to flow regime disturbance, were chosen to characterize the biological groups with regard to their abilities to cope with the changes in flow regimes (additional information about selected traits for each biological group presented in Appendix A – Table A.3; a detailed list of trait acronyms used in the analyses is provided in Appendix A – Table A.4).

Based on the mentioned matrices, the following data analyses were intended to assess the responses of multiple biological elements to different regulation types, responses to a regulation gradient and the existence of any effects of connections between these main effects. All the analyses were performed in the R environment (R Core Team, 2019).

### 2.2.1 Functional diversity and trait analysis

Based on the constructed matrices, RLQ and fourth-corner analyses were performed to assess the potential relationships between traits and the environment, therefore ascertaining possible environmental filtering by species traits. Together, these are two of the most commonly used approaches for exploring trait-by-environment relationships (Leibold and Chase, 2018) and are currently considered to constitute the most integrated way of analyzing these associations (Kleyer et al., 2012). Additionally, plant community functional diversity is considered to be related to disturbance (Biswas and Mallik, 2010; Biswas and Mallik, 2011; Kershaw and Mallik, 2013). RLQ is a multivariate coinertia analysis that relates multiple datasets of species, their traits and environmental variables. It identifies the co-relationships between these datasets by computing a new environment *versus* trait matrix in a PCA-esque fashion, producing a graphical summary of the main structures along orthogonal axes. To perform the mentioned analysis, matrices need to have been previously treated by principal component analysis (matrix R), Hill and Smith analysis (matrix Q, as it contains a mix of numerical and categorical variables) or correspondence analysis (matrix L). RLQ analysis was performed using the *rlq* function from the *ade4* R package. The Fourth-corner analysis tests every possible single association between traits and environmental variables with a corrected type I error. Fourth-corner analysis was performed using the *randtest* function (with 49999 repetitions to have sufficient power in corrected tests) from the R package *ade4*.

Subsequently, functional diversity indices were computed using the *dbFD* function from the *FD* R package. This function uses Gower's distance to enable the use of both continuous and qualitative functional traits (Schleuter et al., 2010) and a principal coordinates analysis (PCoA) to return axes that are then used as traits for computing

functional diversity. Functional diversity describes the roles of biological elements in an ecosystem while considering complementarity and redundancy of co-occurring species (Díaz and Cabido, 2001; Petchey and Gaston, 2006). It is a concept that is increasingly being used in ecological research (Schleuter et al., 2010), having the potential to reveal biological community structuring (Mouchet et al., 2010), and considered a better indicator of ecosystem productivity and vulnerability than species diversity (Biswas and Mallik, 2011; Hu et al., 2014). Nevertheless, there is not an index meeting all the criteria for general use (Villéger et al., 2008), and thus several must be examined. The considered functional diversity indices were multidimensional distance-based indices, namely, functional richness (FRic), functional evenness (FEve), functional divergence (FDiv), functional dispersion (FDis) and Rao's quadratic entropy (RaoQ). Such a panoply was chosen to encompass the assessment of all the functional components, as advised by Mouchet et al. (2010). Furthermore, Mason et al. (2013) demonstrated that functional diversity indices measuring functional richness or functional divergence are required for analyzing assembly processes along stress gradients. The FRic index quantifies niche space occupied by the inhabiting species, and low values indicate unutilized potentially available resources (Mason et al., 2005). It is correlated with species number (does not account for abundance), although species clustering in the community may influence it otherwise. The FEve index measures how mean functional traits are regularly distributed within the occupied trait space, providing insight on how resources are used. Thus, it is a measure of productivity, reliability and vulnerability to invasion. Low values of FEve indicate overrepresentation of particular traits and the near absence of others, resulting in low resource usage with large vacant trait space, and therefore leaving the community vulnerable to invasion (Karadimou et al., 2016). The FDiv index measures the variance of the species function in the trait space, therefore

indicating the degree of resource differentiation and thus competition (Mason et al., 2005) or the predominance of extreme species. High FDiv specifies high resource differentiation, this is, low resource competition. Accordingly, high FDiv values may indicate increased ecosystem functioning due to greater efficiency of resource use (Mason et al., 2005). The FDis index is a measure of species scattering in the trait space and has been suggested as an index of beta diversity (Anderson, 2006). It is calculated as the mean distance of each species to the community centroid, weighted by abundance. High FDis values represent greater functional dissimilarity and consequently a broader range of responses to environmental disturbances (Laliberté et al., 2010). As a result, this index provides a functional diversity measure in which response to environmental gradients is not influenced by species number, while considering both the occupied volume and distribution in the functional space (Laliberté and Legendre, 2010). The RaoQ index is a measure of diversity defined by Rao (1982), considering the proportions of the abundance of species in a community and the dissimilarity among them. It is an improvement compared with the previous measures of functional diversity (Botta-Dukát, 2005) and a capable indicator of disturbance (Péru and Dolédec, 2010). It has the advantage of being influenced by both environmental and habitat filtering and is thus suitable for testing both trait convergence due to environmental filtering and trait divergence due to limiting similarity (Botta-Dukát and Czúcz, 2016). High RaoQ values indicate communities with simultaneously high trait differentiation and high species abundances.

### 2.2.2 Biological diversity analysis

Measuring biological diversity is another tool for quantifying this very complex phenomenon. Biological diversity indices were calculated using function *diversity* from

the R packages *vegan* and *diverse*. Biological diversity at each sampling site was assessed in terms of  $\alpha$  diversity, the average species diversity within sample sites (Whittaker, 1972). The diversity indices considered included Richness (S), Shannon-Wiener diversity ( $H'$ ), Simpson diversity (D), Pielou's Evenness (J) and Berger-Parker (B). Richness is the number of species per sample. It does not account for species abundance and gives the same weight to rare and abundant species. It is also the simplest and most popular measure for quantifying diversity (Magurran, 2004). The Shannon-Wiener index is most commonly used as an index of diversity in ecological studies and incorporates both richness (species number) and evenness. Higher values of  $H'$  represent richer and even communities. Simpson's index is a measure of dominance and provides a probability that two individuals drawn at random from an infinitely large community belong to different species. It increases as species richness and evenness decrease. Evenness is a measure of the relative abundance of the different species composing the species richness of a sample, hence providing a measure of the diversity based on the level of community species dominance. The Berger-Parker index (Berger and Parker, 1970) has already been considered as a practical and effective tool to measure the impacts of human disturbance on ecosystems (Caruso et al., 2007) and is also a measure of dominance. Accordingly, high values of this index indicate uneven community distributions dominated by few species and consequently, disturbed communities.

Pearson correlations were computed between biological diversity indices and environmental data to assess relationships between diversity measures and environmental pressures. Principal component analysis (PCA) of the correlation matrix was performed to determine which environmental variables could better differentiate the

biological diversity indices and, as result, assess how environmental variables could influence the biological diversity of the sampling sites.

### 2.2.3 Responses of biological and functional diversity indices to regulation

Responses of biological and functional indices to river regulation gradients were analyzed using linear regression models. This is a traditional useful approach to decrease the complexity of data sets (Bernhardt-Römermann et al., 2008) and a good exploratory technique to reveal which environmental drivers and traits are most important in performance filtering (Webb et al., 2019). For this, the statistical assumptions of linear modeling, namely, linearity and independence between variables and normality and homoscedasticity of the residuals, were assured through visual examination of the regression diagnostic plots. Spatial autocorrelation was also ruled out by testing every index based on Moran's I statistic. This test revealed that in approximately 90% of the cases the null hypothesis (null spatial autocorrelation present) could not be rejected with a 5% confidence level and the remaining 10% were barely significant in general. Linear models were created for biological and functional diversity indices as a function of the degree of regulation (DOR), as calculated by Lehner et al. (2011). The DOR stands for the ratio of the river's annual flow volume that is retained by the reservoir upstream of a particular river's cross-section and gets smaller as smaller is the proportion of this retained flow volume. Thus, the further downstream from the dam, the less is the DOR. This index has already proven its usefulness for relating river quality with flow regulation (e.g., Grill et al., 2015; McManamay et al., 2016; Lozanovska et al., 2020). The option of adopting linear models is debatable as many processes and biological responses in rivers do not assume linear relationships. Notwithstanding, this study tries to use a robust and common approach to every

biological community, more in a holistic fashion of the fluvial ecosystem, and therefore focus on the general trend of data. Furthermore, this option is supported by the linearity assumption previously assured.

In order to ascertain the effects of the flow regulation type on the response of the indices, an analysis of covariance (ANCOVA) was first performed to determine the variation between and within case studies (rivers), to understand if the responses of dependent variables (biological and functional diversity indices) to the independent variable (DOR) were significantly different between each case study, and thus if the sampling sites should be treated together or in parallel. Afterwards, the linear model was assessed by the F-test of overall significance reflecting whether models including biological and functional diversity indices provided a better fit than the intercept-only model, retained for further analysis.

#### 2.2.4 Ecosystem connections

Correlations between biological and functional diversity indices were performed to assess connections between biological groups. Pearson correlations were computed between biological and functional diversity indices of every biological group, and absolute correlations above 0.7 were highlighted. A network graph was used to better visualize these relationships.

### 3. Results

A total of 31 sampling sites were surveyed in Lima (17) and Alva (14) case studies. Herein, we identified 19 riparian woody species (trees, shrubs, lianas) of which the most



widespread species were alder (*Alnus glutinosa*) and gray willow (*Salix atrocinerea*). A total of 42 macrophyte species were recorded, from which the most common (present in more than 60% of sites) were *Apium nodiflorum*, *Mentha aquatica*, *Lythrum salicaria*, *Juncus effusus* and *Myriophyllum spicatum*. Surveyed bryophytes totaled 20 taxa and were composed mainly by species of turfs and mats, native and common in Northern Portugal riverbeds, considered either hygrophilous lotic or lentic, rarely rheophilous taxa but mostly sciophilous hygrophilous taxa (e.g., *Plagiomnium affine*, *Trichostomum brachydontium*, *Kindbergia praelonga*). A total of 15 macroalgae were identified in 19 sites. Two out of 14 in Alva study site had no macroalgae, while in Lima there were ten out of 17 sampling sites without any macroalgae. The most common taxa were those preferring slowly flowing and stagnant waters: *Sporogyra* sp. and *Oedogonium* sp., followed by rheophilic *Lemanea* sp., which was also collected in 12 sites. The diatom community was the most biodiverse group, with 154 species identified in the surveyed sampling sites. The most common species was *Achnanthydium minutissimum*, present in all sampling sites, followed closely by *Eolimna minima*, *Gomphonema parvulum*, *Gomphonema rhombicum*, *Navicula notha* and *Encyonema silesiacum*. In both case studies, macroinvertebrate sampling resulted in the taxonomic classification of 80 families comprising a total of 71,854 individuals. Among these, the most representative taxa belong to Ephemeroptera (*Baetis* sp., *Ephemerella* sp. and *Caenis luctuosa*); Diptera (Chironomidae and Simuliidae); and Plecoptera (Leuctridae).

### 3.1 Functional diversity and trait analysis

The RLQ analysis provides a great deal of information regarding the considered biological elements (Figure 2; Appendix B – Table B.1). The cumulative projected inertia of the RQL analysis ranged for all the biological elements from 74 to 92% on the

first two axes. Specifically, for the R, Q and L matrices, the RQL analysis was able to explain in the first two dimensions between 65 and 91% of the variation for environmental variables (R matrix), 64 and 84% for traits (Q matrix) and 54 and 60% for species (L matrix).

The most correlated environmental variables were, in the first two dimensions and for every biological element, regulation and habitat variables (Appendix B – Table B.1). The latter also resulting from regulation, this shows a clear influence of river regulation in differentiating sampling sites and their biological communities, which is noticeable by the clear distinction between sampling sites located in regulated and free-flowing circumstances for every biological element, except for macroinvertebrates (Figure 2 – R row scores and R canonical weights) and the eigenvalues for every biological element (Figure 2 – lower right bar plots).

Of the biological element traits (Figure 2 - Q Canonical weights), increased regulation leads to the presence of riparian species with higher canopy heights, deeper rooting systems, and prevalence of reproduction by seeds and rarely vegetatively. For macrophyte vegetation, reductions in regulation lead to communities with less fragmentation, greater emergent character (i.e., tolerance to submersion/drought periods) and less hydromorphic leaves. For bryophytes, this analysis reveals that whenever regulation decreases, bryophyte communities generally show fewer competitive perennials and dendroid life forms and more perennials. Regarding macroalgae, decreases in regulation tend to result in communities with decreased oospore or zygospore formation, less very large biovolume and less drifting. In the case of diatoms, decreases in regulation lead to communities with reduced size and with a high proportion of low-profile species. For macroinvertebrates, in this case, finer sediments and increased mean flow velocities were related to greater nymph aquatic

stage, lower egg aquatic stage and greater free clutches reproduction mode, while lesser regulation intensities resulted in lower life cycle >1 year, greater life cycle <1 year and lower 5 to 10 mm potential maximum size.

Additionally, for every biological element, except for macroinvertebrates, the permutation tests reveal significant relationships between species and the environment, attesting that species distributions among the sampling sites were influenced by the environment. On the other hand, for all biological elements, the effects of traits on species were not significant, and thus the taxa-trait (L-Q matrices) relationships were not significant. The two tests together showed that the existing species distributions are a result of environmental variables and not their traits, i.e., the assemblages are controlled by the environmental conditions, namely, river regulation.

The fourth corner analysis also confirms the existence of significant relationships between environmental variables and species with particular traits (Figure 3). In this analysis, the randomization tests confirm significant relationships between environmental variables and traits for every biological element, except for bryophytes. This result matches the previous RLQ results, suggesting that a combination of stressors, instead of just a single stressor, may be acting on a combination of traits.

### 3.2 *Biological diversity analysis*

The PCA of the correlations between biological diversity indices and environmental variables was able to explain 49% of data variability with the first two axes and 61% in the first three. Environmental variables most highly correlated ( $>|0.70|$ ) with the first two dimensions were primarily related to habitat conditions and river regulation. This analysis reveals that biodiversity indices may be responding differently to particular

influencing factors. For instance, Simpson and Berger-Parker indices are clearly associated with the influence of river regulation variables, Richness seems to respond more to habitat conditions, while the Shannon-Wiener index is related to habitat conditions and water quality. Evenness, on the other hand, did not show a clear trend in relation to any variable.

### 3.3 Responses of biological and functional diversity indices to regulation:

When analyzing the linear relationships between indices and the regulation intensity portrayed by DOR, the ANCOVA results show that, for a confidence level of 95%, effect of river type was only significant for a few indices of all the biological groups. Even many of these are in limbo, with significance depending on the chosen confidence level. Accordingly, in general, river type does not seem to influence greatly the biological elements considered (except for macroalgae), and, for the sake of clarity, the adopted approach was the same for every biological group, which is the following data analyses disregarding river types (Table 1).

The biological and functional diversity indices changed according to DOR for the considered biological elements, but these changes were only significant (confidence level of 95%) for some indices of the biological groups. Based on these results, macroinvertebrates seem to be the biological element with more significantly responsive indices, followed by riparian vegetation and macroalgae. On the other hand, none of the bryophyte index models were useful, revealing that this community does not change significantly along with the DOR in terms of biodiversity or functional diversity. Looking to the considered indices, Richness was the one with more responsive biological elements followed by Simpson, Berger-Parker, FEve, FDiv and RaoQ (Table 3).

Looking at the linear models of biological and functional diversity indices according to DOR, it is noticeable that the biological groups respond in the most varied ways, many times together in general in a concerted way (e.g. Richness and FEve) other times completely independent (e.g. FDiv and RaoQ; Figure 4).

Although many biological and functional diversity indices did not respond significantly to DOR, many of those were strongly correlated ( $>|0.70|$ ) with indices of other biological elements with a significant response to this variable. In general, within biological groups, there were several highly correlated indices, particularly in macrophytes, bryophytes, macroalgae and diatoms. Nevertheless, there were also several intergroup correlations, principally riparian with bryophytes and these two with macroalgae. In particular, of the indices significantly responsive to DOR, riparian FDiv was highly correlated with bryophyte FRic and FDiv, macroalgae RaoQ with bryophyte FEve and macroinvertebrate Berger-Parker and FDiv with bryophyte FEve (Figure 5).

#### **4. Discussion**

This study was outlined to assess the effects of longitudinal gradients of river regulation on several fluvial biological elements. The authors are aware of previous literature addressing this topic, but consider that previous efforts were never so focused on the relief of regulation below dams and, simultaneously, considering such a wide range of biological elements and their connections. For instance, Cortez et al. (2012) presented an interesting paper regarding the effects of a river regulation gradient on water quality, benthic macroalgae and macroinvertebrates, but not specifically along the same river downstream of a dam and with much less sampling effort. Nevertheless, these authors concluded that there was a regulation gradient to which biological communities

responded, particularly macroinvertebrates. Ellis and Jones (2013) performed a thorough literature review on this topic, collecting the results of earlier studies to provide evidence bearing on the serial discontinuity concept predictions regarding physical, chemical and benthic macroinvertebrate community recovery downstream of dams. Lozanovska et al. (2020) determined minimum levels of river regulation significantly affecting biological communities, but only for riparian, macrophyte and bryophyte groups. Thus, this study is a valid step forward, with a more comprehensive and in-depth examination of fluvial ecosystems. Furthermore, this study analyzes both biological and functional diversity of the surveyed communities, in order to understand not only how species composition is affected by regulation but also how it affects ecosystem functioning.

Regarding functional diversity and trait investigation, the RLQ analysis was able to explain a great amount of variation in the data and showed that regulation was, in general, the most important factor differentiating biological communities in both case studies. It is true that habitat variables were also highlighted in this analysis, but habitat is highly dependent on the flow regime (Bunn and Arthington, 2002) and therefore does not invalidate the governing capacity of river regulation on aquatic and riparian communities. First, this relationship substantiates the sampling design for this analysis; second, it upholds previous research determining river regulation as the main factor threatening river health and biodiversity (e.g., Allan and Castillo, 2007; Arthington, 2012; Bunn and Arthington, 2002). Nevertheless, macroinvertebrates were found to be influenced by a more complex discriminating variable network, which has been already supported by previous studies (e.g., Feio et al., 2005; Fonnesu et al., 2005; Graça et al., 2004) indicating that river regulation may influence biota indirectly through habitats.

The permutation tests showed that only environmental conditions are influencing biological communities and not the traits. This reveals an environmental control of river regulation over species assemblages and demonstrates that community species composition actually changes under the effect of an environmental gradient and not due to the influence of trait composition. Furthermore, it reveals that fitness-related traits are shaping the suitability of species to the living conditions determined by regulation at each sampling site. Accordingly, this seems to confer an artificial environmental filtering (Keddy, 1992) that river damming imposes on downstream fluvial communities, in which flow regulation is acting as a selective force controlling species assemblages characterized by particular common phenotypic traits that control fitness in the context of abiotic factors (Kraft et al., 2015). This is, in fact, consistent with what Radinger et al. (2019) previously found for fish fauna.

The phenotypic similarities reflecting regulation tolerance among community members were highlighted by the fourth-corner analysis and were present for every biological element except for bryophytes. Peculiarly, Downes et al. (2003) and Englund et al. (1997) who investigated this relationship in particular came to the same conclusions. The fact that bryophytes do not exhibit specific traits that stand out as regulation fitness-related features may have to do with their relatively low frequency and abundance in the overall sampling process, reflecting the lack of suitable (micro-)habitat in the type of rivers that are strategically regulated in Portugal. Otherwise, the biological elements showed more or less significant relations between environment and traits, indicating that the environmental gradient resulting from river regulation probably affects biological groups differently. Nonetheless, for all the remaining biological groups, significant relationships were in general for regulation and habitat variables. Macroinvertebrates in particular, and in contrast to the other biological groups,

presented more significant relationships between traits and habitat variables rather than for regulation variables, which validates the previous results of RLQ analysis. Again, this must have to do more with the indirect influence of this disturbance on the habitat, to which macroinvertebrates are highly adapted (Feio et al., 2005; Serra et al., 2019).

In terms of biological diversity, the PCA of the correlations between biological indices and environmental variables reveals again that species communities are greatly influenced by habitat and regulation. Although not so powerful in explaining data variability, this analysis showed that the different indices may respond differently to particular environmental conditions. Simpson and Berger-Parker indices are influenced by regulation, whereas Rao and Richness respond more to habitat ecohydraulic conditions. There is also the case of the Shannon-Wiener Index, which additionally seems to respond to water quality. This confirms that there are particular effects of the environmental variables on specific facets of diversity (Friberg, 2010; Li et al., 2019), provides complementary information about various ecological processes (e.g., Corbelli et al., 2015; Heino and Tolonen, 2017) and highlights the appropriateness of particular indices for measuring different aspects of river flow regime regulation.

Regarding the responses of biological and functional indices to river regulation, the results show that in general the different types of river regulation, and consequently the quantity of water withdrawn from the system, did not significantly affect either biological or functional diversity indices. The exception is for macroalgae, whose biological diversity indices ANCOVA results were all significant. Although macroalgae, by having clear preferences to water flow (e.g. rheophilous and rheobiontic species; Rott and Wehr, 2016), can be sensitive to changes in flow regime; some studies (e.g., Downes et al., 2003) reveal that flow regulation may not affect the occurrence of taxa directly, but this is most likely an effect of other important habitat factors, like



dominating substrate. Lange et al. (2016) have also shown that factors related to land use, such as farm intensity, have greater impact on stream algae than does water abstraction. Effectively, the biology between rivers may be different, but this is attributed most often to a range of variables such as water trophy, habitat quality and riparian land use (e.g., Gieswein et al., 2017) and not to river regulation itself, which exerts influence through other components of the flow regime rather than the amount of water (Poff et al., 1997). The decision to include all sampling sites together in the analysis, regardless of river regulation type, can, in fact, impair the particular analysis of macroalgae biodiversity indices, but the authors' final decision was to lose some detail at the biological group level but gain greater robustness at the ecosystem response level.

Most of the biological and functional diversity indices revealed some level of change according to DOR; despite that, only about a third of the indices showed significant changes for a confidence level of 90%. This may indicate that the considered regulation intensity range may not have been broad enough to reach significance in the different communities or that a particular index is, in fact, unaffected by DOR. The results suggest that biological elements present different sensitivities to regulation intensity, becoming most pressing with the focus of necessary restoration measures. For instance, macroinvertebrates and riparian vegetation were significantly responsive to DOR for half or more of the indices, whereas bryophytes were responsive for none, and macrophytes and diatoms only one out of ten. The numerical lack of responsiveness of bryophytes to DOR may once again be an artifact of low sampling and low presence of this group of plants in these studied rivers' reaches.

Moreover, recent scientific research has been highlighting the sensitivity of the various biological groups and traits to river regulation (e.g., Abati et al., 2016; Aguiar et al., 2018; Rivaes et al., 2015; Sabater et al., 2018; White et al., 2017). This work goes

beyond that, revealing the recovery trends of the biological groups facing a decrease in regulation below dams, and enables comparisons among them. For instance, macrophyte communities were composed mostly of rooted aquatic macrophytes and emergent species, the latter being tolerant to periodic water drawdowns and waterlogging. In addition to flow velocity, the amount of fine sediments is a key factor mediating the persistence of these communities in rivers (Green, 2005). In turn, macrophytes modify the flow patterns at the reach scale, contributing to the construction of mesohabitats by trapping fine sediments (Gurnell et al., 2006). In our study, we have confirmed that species with hydromorphic leaves (truly aquatic species) are related to fine sediments (sand, silt), and this may surpass the constraints of regulation (water depth, flow velocity, hydropeaking). Nevertheless, it is difficult to disentangle these feedback effects of sediment and regulation dynamics on these fluvial communities. On the other hand, the existing knowledge on riparian woody communities has shown the numerous effects of regulation on the establishment and colonization of pioneer communities, with consequences for short- and long-term riparian ecosystem functioning (Aguiar et al., 2018; Benjankar et al., 2012; Garófano-Gómez et al., 2012; Gurnell et al., 2012; Rivas et al., 2015).

Additionally, the biological and functional diversity indices present different levels of response to the same regulation gradient. This raises yet another question, with regard to the sensitivity of the different indices to regulation and therefore their particular capacities for assessing different levels of regulation intensities or gradients. This analysis also provides very useful information regarding this, indicating which diversity indices may be most favorable for determining the impact of river regulation on aquatic and riparian communities. Accordingly, Richness was the index with more significant responses to DOR from the considered biological elements, providing significant

responses for half of the biological elements and coinciding with the most responsive ones, namely, macroinvertebrates and riparian vegetation. This agrees with Kuiper et al. (2014), whose analyses focused on floodplain wetlands, reached the conclusion that species richness is related to the degree of hydrological alteration. Thus, Richness appears to be the most interesting as a single measure for general fluvial ecological quality facing river regulation. Additionally, this is consistent with the WFD approach, where Richness is one of the most used and efficient metrics (e.g. Pont et al., 2020; van de Bund, 2009). Nevertheless, a set of indices may provide a more encompassing analysis of the ecological quality of biological elements determined by river regulation (Villéger et al., 2008).

A joint analysis of multiple indices can provide another view of ecosystem functioning. For instance, Richness decreased along with DOR for every biological element except for macrophytes. This strongly suggests a niche limitation situation where strong biotic interactions are present and restraining the co-occurrence of more species in the habitat (Götzenberger et al., 2012). At the same time, the Simpson index increased along with DOR for riparian, macrophytes, bryophytes and macroalgae. Analyzing both indices together, this may indicate for riparian, bryophytes and macroalgae that increased river regulation causes the loss of more sensitive species and increasing dominance of more regulation-resilient species that can occupy the vacant niches. The existence of diverse strategies of niche occupancy by native and exotic species can partly explain the observed trends. In our study, we observed the fragmentation of the riparian zones by invasive *Acacia* species (especially downstream of Fronhas dam, Alva River) with consequences for the reduction of the overall biodiversity. Nadal-Sala et al. (2017) also reported in Mediterranean riparian forests higher growth-based water use efficiency of cosmopolitan tree species relative to alders and ashes, which are more dependent on

phreatic connections that were partially lost in rivers impaired by reservoirs. Drought-like flow conditions induced by reservoirs reduce the abundance of obligate riparian tree species and can facilitate species with various dispersal strategies, besides hydrochory (Aguiar et al., 2018). On the other hand, turbinated flows and hydropeaking can also filter out species and change the successional stages of riparian plants' establishment by uprooting and reducing germination and colonization by pioneers (Bejarano et al., 2020; Rivaes et al., 2015). On the other hand, both increasing macrophyte indices could indicate that macrophyte growth is likely promoted by both higher nutrient availability and greater sediment loads in the novel environments or regulated reaches. In fact, we observed positive relations with macrophytes and fine sediments, which facilitate the establishment of emergent species such as *Juncus sp.*, *Typha sp.* and *Carex sp.* Regarding the increasing cover of aquatic macrophytes under regulation, the most common species *Myriophyllum spicatum* and *Ranunculus peltatus* increase in cover for different reasons. The first prefers shallow, moderately turbid waters and nutrient-rich sediments, while *Ranunculus peltatus* growth is promoted by still waters and stressful environments that alternate flooding and drying (Lozanovska et al., 2020). For macroalgae, moreover, it seems that it is a result of clear preferences of specific taxa to flow conditions and not only to the availability of river zones or niches (Rott and Wehr, 2016). Additionally, the clear differences in macroalgae occurrence in the two analyzed catchments showed that a lower amount of flowing water but with more stable flow conditions (Alva) constitute much more suitable conditions than flow fluctuations below a dam (Lima).

However, it is hard to distinguish environmental filtering from biotic interactions like competition (Germain et al., 2018; Kraft et al., 2015). The Berger-Parker index decreases for every biological group except for diatoms and macroinvertebrates. This

index entirely ignores rare species and indicates that diatoms and macroinvertebrates are dominated by the most common species, therefore leading to uneven communities in increased regulation circumstances. For functional diversity, FEve reduction indicates that for macrophytes and macroalgae there is an imbalance of these communities with consequent low resource usage and large vacant niche space, making them vulnerable to invasion (Mason et al., 2005). This meets the outcome of the Simpson index for macrophytes, confirming that river regulation allows for an increased available niche space for this biological element. This is also indicative of increased pressure of environmental filtering (Cornwell et al., 2006). FEve increased significantly along regulation gradients for riparian and macroinvertebrates, revealing that at least these two communities increased in functional stability with a consequent uniformity of species traits, and, thus, although abundance declined, the trait composition was still balanced and retained sufficient resilience to trait invasion. FDiv decreased for riparian, macrophytes and bryophytes, revealing low niche differentiation and high resource competition (Mason et al., 2005). This happens when the most abundant species present functional trait values that are close to the center of the functional trait range and is once more indicative of environmental filtering (Götzenberger et al., 2012), due to the exclusion of traits less well-adapted to the local conditions (Botta-Dukát and Czúcz, 2016). On the other hand, for macroalgae, diatoms and macroinvertebrates, river regulation determines the opposite trend, meaning that the most abundant species present extreme functional trait values (Villéger et al., 2008), an evidence for limiting similarity (Watkins and Wilson, 2003) where biological groups coexist through the resource division with some degree of niche overlap (but see Szabó and Meszéna (2006) for a better understanding). Indeed, this appears to be more pronounced between macroalgae and the other two together, as diatom and macroinvertebrate indices always

present the same trend, while often for macroalgae the trend is the opposite. Finally, riparian and bryophytes RaoQ decreased, while this index increased for the remaining biological elements, indicating a reduction in trait differentiation and low species abundance for these biological elements, again a signal of environmental filtering, and the opposite for the remaining biological elements.

The results also highlight several correlations between biological and functional diversity indices, not only within groups but also between. Particularly, changes in riparian indices are highly correlated with bryophytes and bryophytes with macroalgae. Although correlations may not correspond to interactions, this may reveal the existence of connections between particular biological groups and substantiates the habitat cascade effects originated by human-driven habitat modifications (Thomsen et al., 2010). Notwithstanding, this is a topic that needs further detailed investigation, so as to fully understand particular interactions between biological groups. For now, considering the high responsiveness of riparian vegetation to river regulation and its high correlations with bryophytes and macroalgae, this can indicate that bryophytes and macroalgae may also be consequently influenced by river regulation. Thus, although bryophytes were not found to be influenced directly by river regulation, further investigation is needed to understand if this disturbance can indirectly influence this community via riparian vegetation. In fact, it is known that bryophytes respond to near-ground microclimatic gradients promoted by riparian woody communities, such as shade and shelter, by scouring in tree trunks (Stewart and Mallik, 2006; Turunen et al., 2019) and thus, tend to increase in forested streams. River bryophytes depend also on substrate stability, which is greatly influenced by the degree of regulation and changes in strong discharge patterns. Life-form and life-strategy diversity is strongly dependent on the balance between disturbance events and scouring processes and the hydrological

permanence and depth variability determines much of the trait richness at the patch and reach levels (Fritz et al., 2009; Vieira et al., 2012).

Overall, despite the great difficulty in distinguishing the direct effects of modified flow regimes from the impacts of land-use change (Bunn and Arthington, 2002), all the results point to solid evidence of river regulation governing the assessed communities. Looking at the biological groups, and based on the previous discussion, there is strong evidence of environmental filtering of overall communities. Furthermore, macrophytes appear to be competing for niche space with the remaining biological groups, while limiting similarity was highlighted for macroalgae, diatoms and macroinvertebrates. This indicates that increasing river regulation tends to eliminate fewer adapted species and decrease a community's richness, while the remaining species face increased interactions like resource competition and limiting similarity, reduced buffering against environmental fluctuations (Tilman, 1996) and decreased invasion resistance (Dukes, 2001). Finally, even for those biological groups that do not respond significantly in a direct way to river regulation, this disturbance may have an indirect impact, as the dense network of biotic connections supporting the fluvial ecosystem can establish such connection through a cascade of effects. This emphasizes the importance of considering the entire fluvial ecosystem when assessing the effects of river regulation on riparian and aquatic fluvial communities.

## **5. Conclusions:**

The sampling design adopted in this research was capable of capturing the longitudinal river regulation gradient existing in the study sites, a basic requirement to support the objectives of this study.

River regulation and habitat variables were demonstrated to be the major factors differentiating biological communities in general.

River regulation promotes an artificial environmental filtering of species assemblages, whose amendments follow the effect of an environmental gradient. This environmental gradient acts as a selective force controlling species assemblages through the prominence of particular phenotypic similarities reflecting regulation tolerance.

The biological communities exhibited particular traits significantly correlated with environmental variables, except bryophytes. Riparian vegetation and macroinvertebrates showed the greatest number of significant correlations between environment and traits.

Except for macroalgae, the biological and functional diversity of the considered communities appear not to be influenced by river regulation typology. Notwithstanding, all biological communities respond even though differently, to a river regulation gradient. Furthermore, the different facets of biological and functional diversity, assessed by the different indices, presented different sensibilities to the river regulation and its disturbance gradient.

Some biological elements are more pressing than others with respect to the focus of necessary restoration measures. In this case, riparian vegetation and macroinvertebrates appear to be the most responsive to river regulation and its gradient.

Biological and functional diversity indices seem to have different sensitivities to regulation effects. Richness was revealed to respond better to regulation for the biological communities in general and appears to be the most interesting single measure for general fluvial ecological quality facing river regulation.

There are high correlations between the biological and functional diversity of different biological groups, which emphasizes the habitat cascade effect promoted by anthropic



disturbances and stresses the necessary holistic perspective when assessing the effects of river regulation on fluvial ecosystems.

In the end, this work supports previous research determining river regulation to be the main factor threatening river health and biodiversity.

### **CRedit authorship contribution statement**

**Rui Rivaes:** Conceptualization, Methodology, Formal analysis Investigation, Writing – Original Draft, Writing – Review & Editing, Visualization. **Maria João Feio:** Investigation, Resources, Data Curation, Writing – Review & Editing, Supervision. **Salomé FP Almeida:** Investigation, Resources, Data Curation, Writing – Review & Editing, Supervision. **Cristiana Vieira:** Investigation, Resources, Data Curation, Writing – Review & Editing, Supervision. **Ana R Calapez:** Investigation, Data Curation, Writing – Review & Editing. **Andreia Mortágua:** Investigation, Data Curation, Writing – Review & Editing. **Daniel Gebler:** Investigation, Data Curation, Writing – Review & Editing, Supervision. **Ivana Lozanovska:** Investigation, Data Curation, Writing – Review & Editing. **Francisca Aguiar:** Conceptualization, Methodology, Validation, Investigation, Writing – Original Draft, Writing – Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.

### **Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1. RQL and fourth corner results.

Biological element	RLQ cumulative projected inertia (first two axes)	RLQ inertia & coertia ratio (environmental)	RLQ trait & coertia ratio (traits)	RLQ inertia & coertia ratio (species)	RLQ most correlated environmental variables with Axis 1	RLQ most correlated environmental variables with Axis 2	RLQ most correlated traits with Axis 1	RLQ most correlated traits with Axis 2	Permutation test Model 2 (p-value)	Permutation test Model 4 (p-value)
Riparian	75%	90%	83%	57%	- DOR - Channel width - Regulated watershed ratio	- Flow regulation ratio - Mean discharge ratio - Sand	- Seed reproduction - Seeds and/or vegetative reproduction - Deep rooting depth	- Other dispersal vector - Hydrochory dispersal vector - Diaspore type	8.0E-04	0.32
Macrophyte	81%	81%	84%	54%	- Mean flow velocity - Sand - Channel width	- Flow regulation ratio - Regulated river length ratio - Regulated watershed ratio	- Fragmentation - Emergent - Hydromorphic	- Annual - Perennial - Elliptical-pinnate or elliptical-rectangular leaves	1.4E-03	0.13
Bryophyte	92%	90%	81%	59%	- Regulated watershed ratio - Regulated river length ratio - DOR	- Mean flow velocity - Cobbles - Sand	- Mat lifeform - Turf lifeform - Perennial life strategy	- Competitive perennial life strategy - Perennial life strategy - Dendroid lifeform	2.5E-02	0.67
Macroalgae	90%	65%	78%	58%	- Sand - Mean flow velocity - Mean discharge ratio	- Regulated river length ratio - Regulated watershed	- Forming oospores or zygospores - Very large biovolume	- Nano biovolume - Gliding - Not forming spores	6.6E-03	0.15





Table 2. ANCOVA results (p-values) to linear relationships of diversity indices and functional diversity indices as a function of regulation intensity when considering or not the factor river type (significance level: 90% . , 95% \*, 99% \*\*, 99.9% \*\*\*).

Biological element	Biological diversity indices				Functional diversity indices					
	Richness	Shannon	Simpson	Evenness	Berger-Paraker	FRic	FEve	FDi v	FDi s	Rao Q
Riparian	0.14	0.26	0.33	0.34	0.56	0.19	0.08	0.63	0.98	0.90
Macrophyte	0.88	0.40	0.31	0.13	0.40	0.99	0.01*	0.18	0.58	0.83
Bryophyte	0.50	0.61	0.88	0.78	0.72	0.16	0.94	0.33	0.40	0.37
Macroalgae	9.96E-05 ***	1.37E-04 ***	3.339E-03 **	3.00E-03 **	0.01 **	0.53	0.69	0.22	0.55	0.94
Diatom	0.43	0.33	0.20	0.38	0.12	0.33	8.46E-03 **	0.20	0.10	0.07
Macroinvertebrate	0.01 **	0.56	0.92	0.26	0.92	2.28E-03 **	0.22	0.77	0.50	0.31

Table 3. Results of the F test (p-values) of linear models between functional diversity indices and DOR (significance level: 90% . , 95% \*, 99% \*\*, 99.9% \*\*\*).

Biological element	Biological diversity indices				Functional diversity indices					
	Richness	Shannon	Simpson	Evenness	Berger-Paraker	FRic	FEve	FDiv	FDiss	Rao Q
<b>Riparian</b>	0.01 *	0.79	0.57	2.93E-04 ***	0.23	4.07E-04 ***	0.03 *	0.02 *	0.37	0.17
<b>Macrophyte</b>	0.09 .	0.45	0.61	0.61	0.67	0.24	0.33	0.70	0.35	0.38
<b>Bryophyte</b>	0.37	0.26	0.90	0.27	0.74	0.73	0.56	0.30	0.20	0.31
<b>Macroalgae</b>	0.50	0.83	0.01 **	0.19	8.24E-03 *	0.11	0.56	0.70	0.04 *	0.09
<b>Diatom</b>	0.19	0.59	0.31	0.81	0.55	0.76	0.59	0.19	0.19	0.05 *
<b>Macroinvertebrate</b>	0.08 .	0.02 *	0.02 *	0.15	0.01 *	2.65E-03 **	0.38	0.06	0.84	0.82

Figure 1. Location of Lima and Alva case studies and arrangement of sampling sites (numbered circles) in relation to the respective dams (white dam locks).

Figure 2. RLQ analysis results for the considered biological elements. Orange and green ellipses cluster regulated and free-flowing sampling sites, respectively. Blue arrows highlight the loadings of the three major correlated environmental variables with the main axis in differentiating sampling sites (1, 2 and 3 relate to the corresponding first, second and third variables mentioned in Table 1). Red arrows highlight the loadings of the three major correlated traits with the main axis in differentiating species (1, 2 and 3 relate to the corresponding first, second and third variables mentioned in Table 1).

Figure 3. Fourth-corner results with significant relationships (randomization test p-value <0.05) between environmental variables and traits highlighted in blue (negative) and red (positive).

Figure 4. Linear models of the biological and functional diversity indices according to DOR.

Figure 5. Correlation analysis between the biological and functional indices of the considered biological elements (positive correlations in green and negative in red. High correlations (>|0.7|) color accentuated with line thickness proportional to correlation).

**CRedit authorship contribution statement**

**Rui Rivaes:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – Original Draft, Writing – Review & Editing, Visualization. **Maria João Feio:** Investigation, Resources, Data Curation, Writing – Review & Editing, Supervision. **Salomé FP Almeida:** Investigation, Resources, Data Curation, Writing – Review & Editing, Supervision. **Cristiana Vieira:** Investigation, Resources, Data Curation, Writing – Review & Editing, Supervision. **Ana R Calapez:** Investigation, Data Curation, Writing – Review & Editing. **Andreia Mortágua:** Investigation, Data Curation, Writing – Review & Editing. **Daniel Gebler:** Investigation, Data Curation, Writing – Review & Editing, Supervision. **Ivana Mozanovska:** Investigation, Data Curation, Writing – Review & Editing. **Francisca Aguiar:** Conceptualization, Methodology, Validation, Investigation, Writing – Original Draft, Writing – Review & Editing, Visualization, Supervision, Project administration, Funding acquisition.

**Declaration of competing interest**

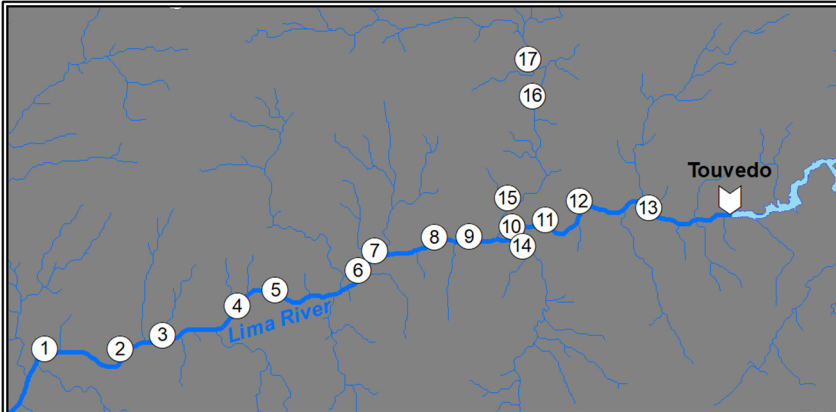
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Journal Pre-proof

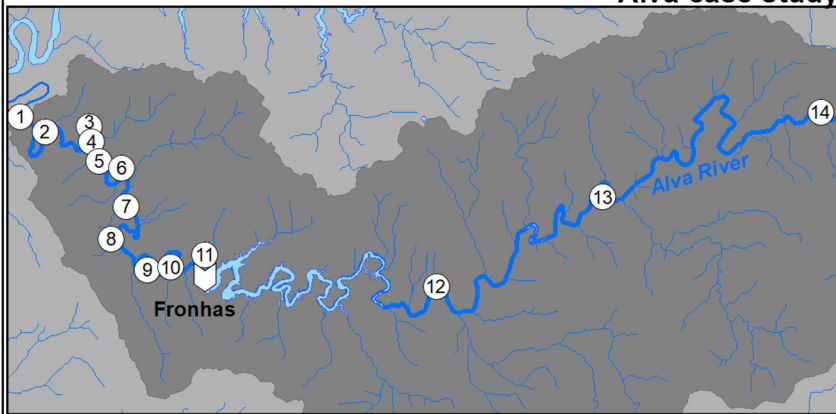
**HIGHLIGHTS:**

- River regulation and habitat are major factors differentiating communities.
- Riparian vegetation and invertebrates most responsive to river regulation gradient.
- Richness most sensitive measure for ecological quality facing river regulation.
- Biological and functional diversities of biological groups are correlated.

Journal Pre-proof



**Lima case study**



**Alva case study**

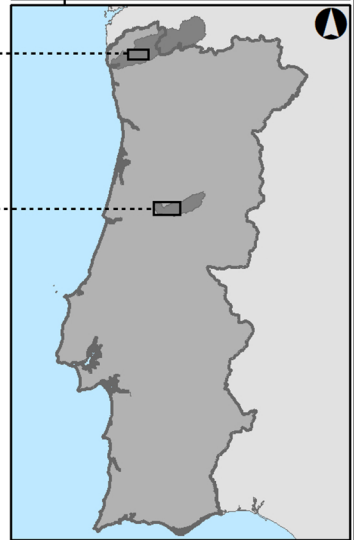
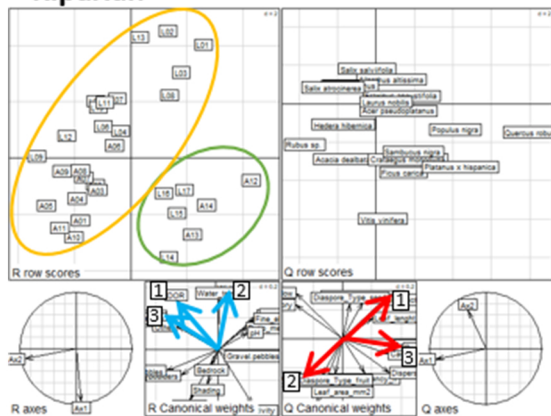
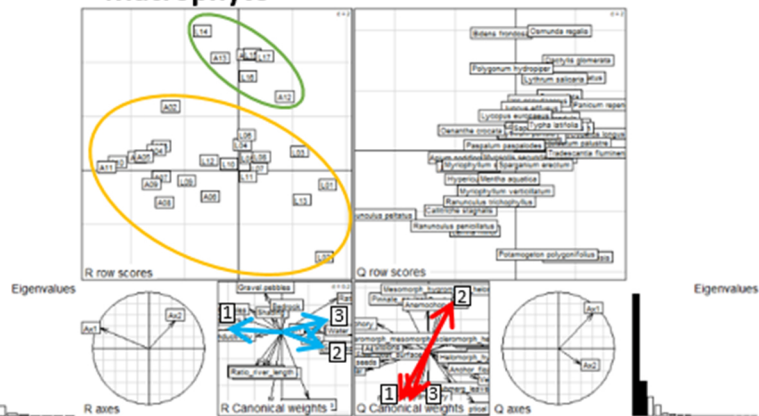


Figure 1

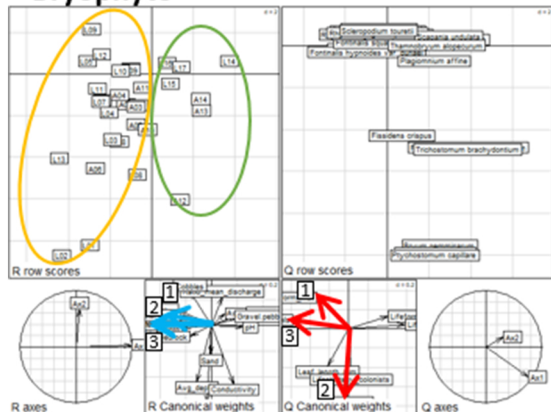
## Riparian



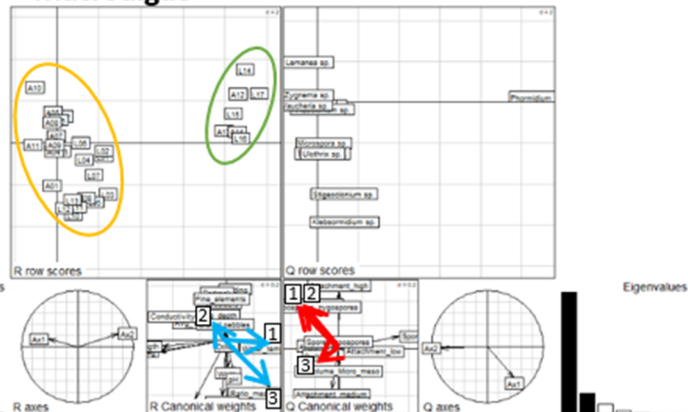
## Macrophyte



## Bryophyte



## Macroalgae



## Diatom



## Macroinvertebrate



Figure 2



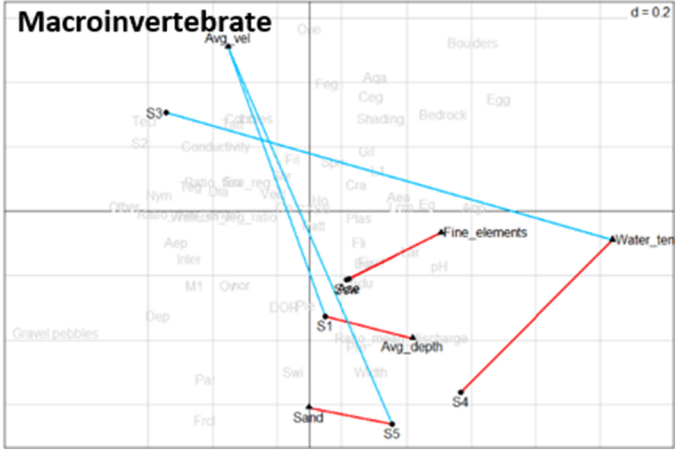
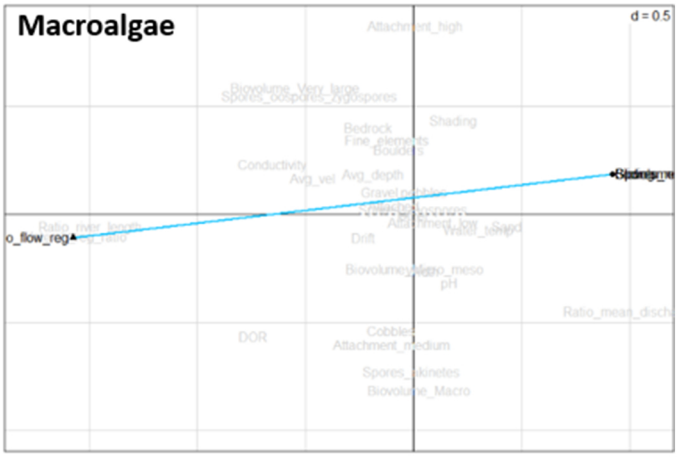
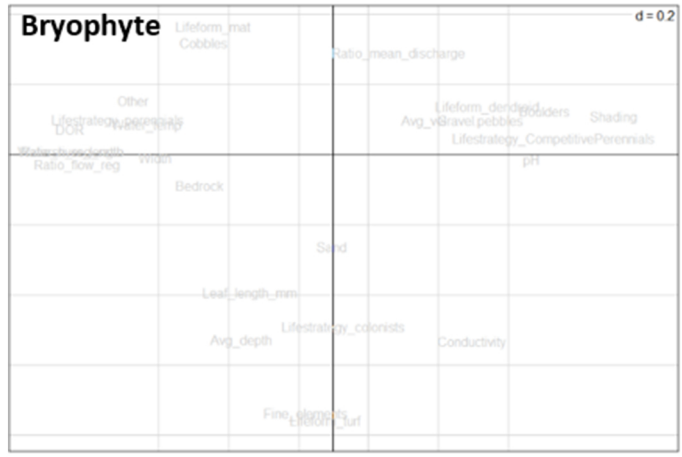
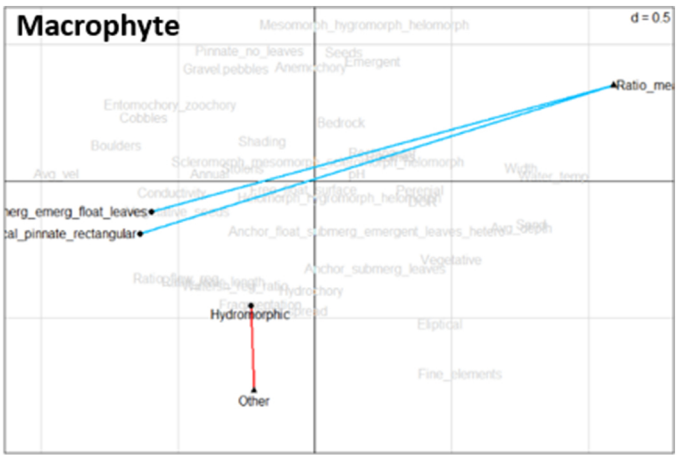
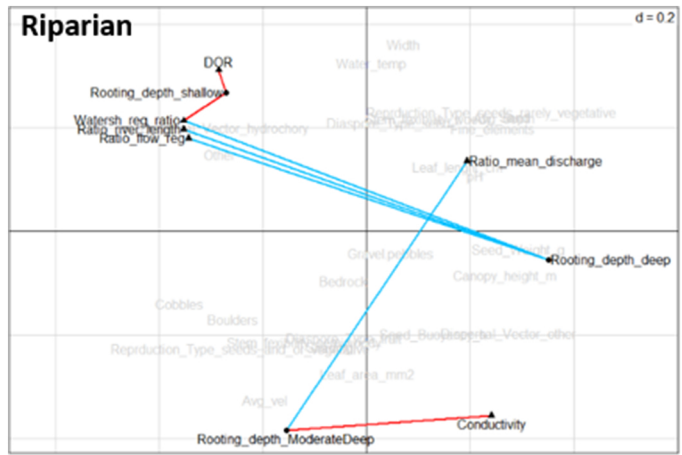


Figure 3

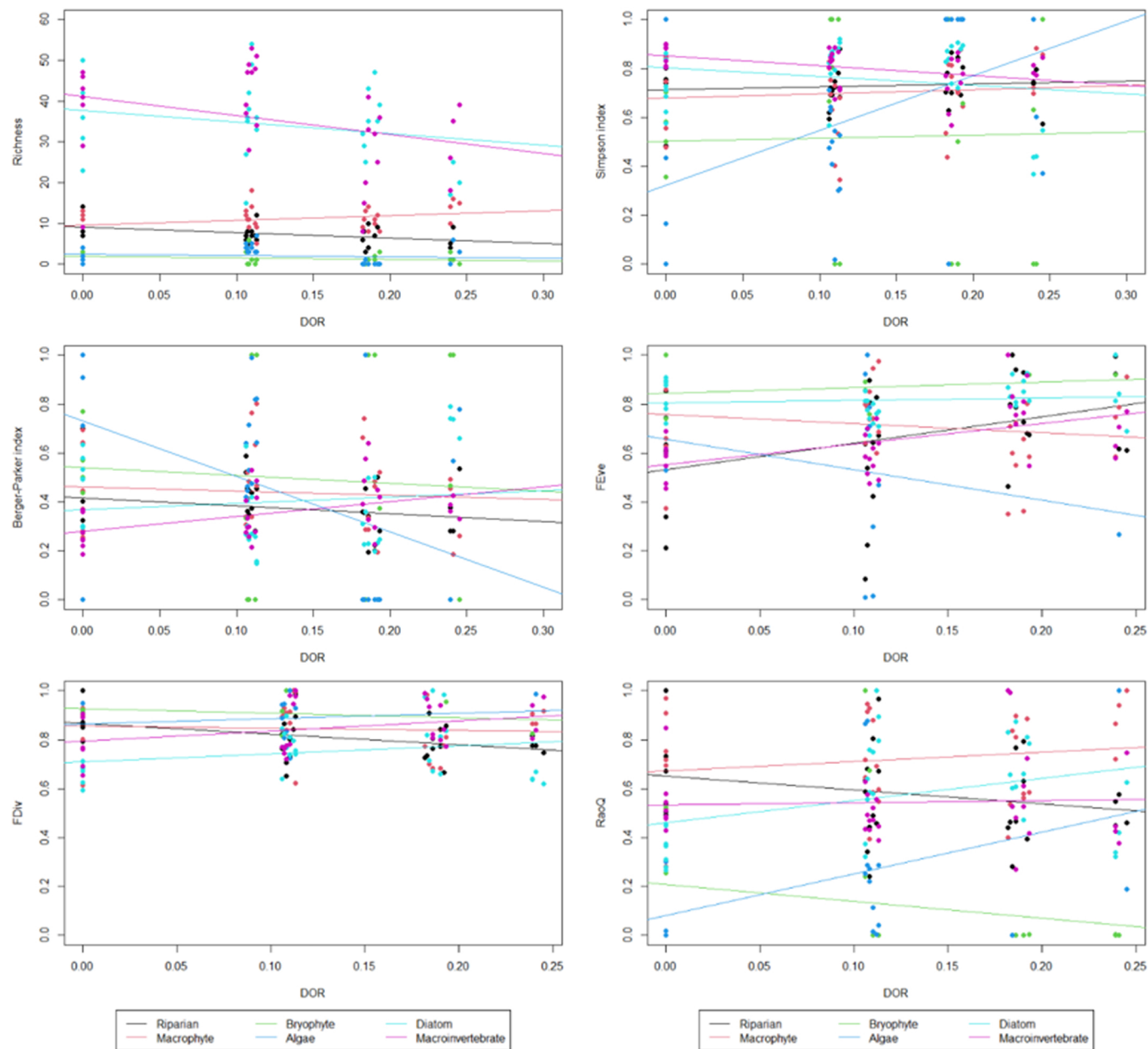


Figure 4

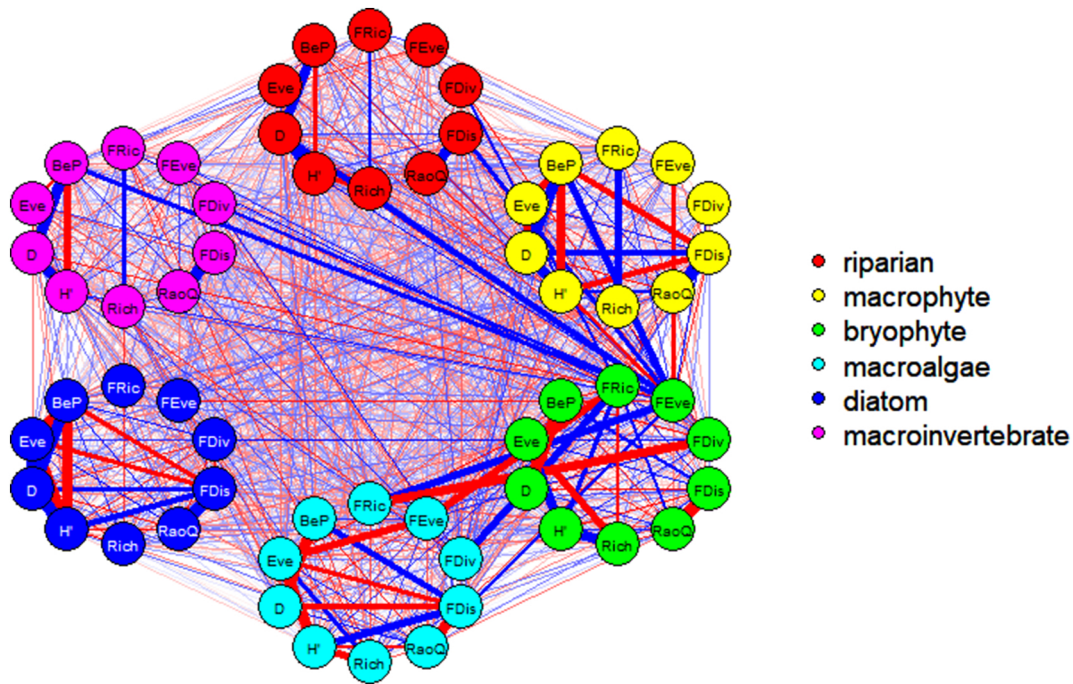


Figure 5