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**Flow regulation increases food chain length through omnivory mechanisms in a  
Mediterranean river network**

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**Keywords:** Dams, Hydrologic alteration, Food webs, Stable isotope analysis, Trophic position.

## Summary

1. Dams fragment river systems worldwide, and Mediterranean-climate rivers, characterized by highly-seasonal hydrographs and adapted biotas, are particularly impacted by flow regulation. Whereas the effects of flow regulation on hydrology, sediment transport and biodiversity have long been examined, responses at the food-web level remain understudied.
2. Environmental variation is a key control of food-web structure. Thus, we predicted that flow regulation would impact food chain length (FCL) via changes in the flow variation regime, and we tested this prediction in a set of flow-unregulated- to completely regulated reaches in a Mediterranean river basin.
3. In each reach we characterized flow variation, together with two other putative controls of FCL (productivity and habitat size). We combined community data with carbon and nitrogen stable isotopes to estimate food chain length, and Bayesian mixing models allowed estimating dietary proportions of consumers.
4. Flow variation was paramount in controlling FCL in the studied river network, and this same control largely explained the degree of top predators' omnivory. Thus, omnivory mechanisms were the main proximate structural mechanism allowing shifts in food-web structure and linking disturbance regimes to FCL.
5. Our results suggest that flow regulation in Mediterranean rivers may impact food-web structure even when no significant changes in community composition are observed. If highly-variable Mediterranean streams become increasingly muted due to flow regulation, stable conditions could enhance intraguild predation and thus lengthen riverine food chains.

**Keywords:** Dams, Dynamic stability hypothesis, Hydrologic alteration, Omnivory mechanisms, Riverine fish, Stream ecology.

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**Introduction**

Dams fragment large river systems across the globe (Nilsson *et al.* 2005), and effects are remarkable in rivers of Mediterranean climates, owing to the high number of dams located in Mediterranean-climate regions (Nilsson *et al.* 2005) and to biota in these rivers being adapted to highly-seasonal hydrographs (Gasith & Resh 1999). Dams impact natural flow and temperature regimes (Lehmkuhl 1972; Poff *et al.* 1997, 2007), sediment transport (Ibáñez, Prat & Canicio 1996; Topping, Rubin & Vierra Jr 2000), and biodiversity both via filtering of life histories (Bunn & Arthington 2002) as well as by facilitation of invasion (Johnson, Olden & Vander Zanden 2008). However, research on flow regulation impacts at the food-web level is still relatively recent, and so far has largely focused on high-order, stable river systems (e.g., Hoeinghaus, Winemiller & Agostinho 2008; Cross *et al.* 2011, 2013). Because smaller rivers are intrinsically more hydrologically variable (Sabo *et al.* 2010) and discharge variation is a key driver of food-web structure (Power, Dietrich & Finlay 1996; Wootton, Parker & Power 1996; Sabo *et al.* 2010), dams in highly-seasonal Mediterranean streams may strongly alter food-web structure.

Food chain length (FCL), or the number of trophic transfers between basal resources and top predators, is a key measure of food-web structure. It has long attracted ecological research (Elton 1927; Lindeman 1942), much of which has occurred in freshwater ecosystems (e.g., Vander Zanden *et al.* 2007; McHugh, McIntosh & Jellyman 2010; Sabo *et al.* 2010; Schriever & Williams 2013). Despite the variety of implications of FCL, still some controversy exists on which are the mechanisms controlling FCL (reviewed in Post 2002a). Three main non-mutually-exclusive hypotheses compete: (i) The productivity or resource availability hypothesis is based on the energy losses that occur with each trophic transfer, and suggests that more productive

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3 47 systems should allow longer food chains than less productive ones (Pimm 1982; Schoener 1989).  
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6 48 (ii) The disturbance or dynamic stability hypothesis is based on the fact that in model systems  
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8 49 longer food chains are more vulnerable and take longer to recover than shorter ones, which  
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11 50 implies that food chains should be shorter in highly variable environments (Pimm & Lawton  
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13 51 1977). Finally, (iii) the ecosystem size hypothesis suggests that because larger ecosystems are  
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15 52 often more heterogeneous in habitat, they should host more compartmentalized food webs and  
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17 53 hence allow for longer food chains than smaller ecosystems (Post, Pace & Hairston 2000). Dams  
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19 54 increase water residence time in the impoundment, mute high flows downstream, and increase  
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21 55 inter-flood interval (Poff *et al.* 2007; Sabater 2008; Aristi *et al.* 2014). Thus, the disturbance  
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23 56 hypothesis of FCL should come into play when assessing food-web structure responses to flow  
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30 58 Within the food web, variation in FCL can only be explained by (i) the addition or  
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32 59 removal of top predators, which changes the identity of the apical predator (additive  
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34 60 mechanisms); (ii) by the addition or removal of primary and secondary consumers, which  
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36 61 changes the trophic position of top predators (insertion mechanisms); or (iii) by changes in the  
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38 62 degree of trophic omnivory by predators (omnivory mechanisms) (Figure 1, adapted from Post &  
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40 63 Takimoto 2007). A change in the incidence or identity of the apical predator (i.e., additive  
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42 64 mechanism) is probably the most evident and widely-discussed proximate mechanism for  
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44 65 variation in FCL (Schoener 1989; Post *et al.* 2000). In contrast, insertion and omnivory  
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47 66 mechanisms have seldom been considered (but see Townsend *et al.* 1998). These mechanisms  
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49 67 assume that FCL may change even if the apical predator remains constant, and thus both may be  
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51 68 assessed by measuring changes in the trophic position of predators (Post 2002a). Within this  
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53 69 context, a few studies have suggested that foraging shifts of predators (Denno & Fagan 2003)  
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and variation in the availability or diversity of consumers (McHugh *et al.* 2010; Young *et al.* 2013) may cause changes in the vertical structure of food webs. Since omnivory is widespread in nature (Polis 1991; Denno & Fagan 2003; Diehl 2003; Thompson *et al.* 2007), omnivory mechanisms may be important controls of FCL across small spatial scales (i.e., when biogeographical effects are minimal), for instance across reaches differing in flow regulation in a single river network.

Here we asked how flow regulation affects FCL in a Mediterranean river network, and which proximate structural mechanisms (*sensu* Post & Takimoto 2007) may explain eventual shifts in food-web structure. To this end we selected 9 stream reaches that were regulated to different extents by several reservoirs and weirs, and characterized the potential environmental controls of FCL (disturbance, productivity, habitat size). We predicted that hydrologic disturbance would be the main control of FCL, with regulation reducing flow variation and thus lengthening food chains (as previously suggested by McHugh *et al.* 2010; Sabo *et al.* 2010). We also tested the strength of evidence supporting additive, insertion, and omnivory mechanisms mediating FCL responses to the environment. According to theory, disturbance differentially impairs the higher levels of food chains (Pimm & Lawton 1977). Thus, when top predators in Mediterranean-climate rivers (usually fishes) recolonize after hydrologic disturbance (floods and droughts), they may be forced to feed disproportionately more on low trophic levels in order to meet their energetic demands. By feeding relatively more on basal resources and less on primary and secondary consumers, their trophic position should decrease relative to that held in hydrologically stable, consumer-rich reaches (Figure 1). If this is true, diet shifts of top predators could be an important mechanism linking flow regulation to FCL in rivers, and evidence on this

could be obtained by comparing energy flow food webs (as opposed to connectance food webs) across sites that differ in flow regulation regimes and FCL.

## Methods

### Study site

Field sampling was conducted during spring and summer 2012 in the Siurana River catchment (NE Iberian Peninsula). The Siurana catchment ranges from 23 to 1000 m.a.s.l. and has an extension of 615 km<sup>2</sup> down to its river mouth, where it reaches the Ebro River (Figure 2). Its land use is characterized by coniferous and evergreen oak forests (45 %), shrublands (24 %) and arable rain-fed lands (22 %). The climate is Mediterranean, with precipitation averaging 590 mm year<sup>-1</sup> (concentrated from October to April). The heaviest rainfall usually occurs in autumn and as a result, there are intense flood episodes during autumn in these water courses, which have either permanent flow regimes with low baseflows or temporary flow regimes. We selected 9 reaches from the two main watercourses in the catchment (Siurana and Montsant), with the last reach being located at the confluence (Figure 2). Flows in these reaches were either unregulated or regulated to different extents by three reservoirs, a weir and a bypass (Figure 2). Each study reach (100 m long, ranging between 3.4 and 9.8 m in width) was centered on a pool and contained at least 2 riffles, one upstream and one downstream of the pool. In this setting, all reaches could potentially share the same faunal pool and could be influenced by similar environmental variables (Table S1). We validated this assumption visually and numerically, with multivariate methods: First, a Principal Components Analysis (PCA) ordered reaches according to their physico-chemical parameters, and reaches did not display any directional pattern (Figure S1). Second, permutation-based models (PERMANOVA and Mantel-type tests) combining physico-chemical variables and biota showed that physico-chemical variation did not

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significantly differ between sections, and did not significantly influence the algae, invertebrate, or vertebrate compositions (see details in Table S2).

Characterizing disturbance, productivity, and habitat size

In order to understand how flow regulation may control FCL in our basin, we first characterized the putative controls of FCL in the light of existing theory (disturbance, productivity, and size hypotheses).

To define disturbance, following McHugh *et al.* (2010) we combined several hydrological and geomorphological measures (Table 1). First, we implemented a distributed hydrological model (TETIS; Francés, Velez & Velez 2007) to characterize hydrology at each reach. The model was supplied with topographical, geological, soil, and land use information, considering the presence of the aforementioned reservoirs and diversions. The model was calibrated and validated using the built-in automatic procedure (Nash-Sutcliffe efficiency = 0.67; see Figure S2 for details). The validation data set consisted of 13 years of daily streamflow data at the inflow of the Siurana Reservoir. After calibration, the model was run for the whole catchment and delivered daily flow series for the 15 years prior to the study (from 1/1/1998 to 30/06/2012) in each of the 9 reaches. We then analyzed those data using the Indicators of Hydrologic Alteration (IHA; Richter *et al.* 1996). With the IHA software v. 7.1 (The Nature Conservancy, Charlottesville, VA, U.S.A.) we computed, for each reach during the entire period, the number of days with small floods (2-year return interval) and with extreme low flows (defined as the 10<sup>th</sup> percentile of daily flows). These metrics (hereafter, *high-* and *low flows*) reflect river rises that overtop the main channel, and drought conditions that may be stressful for organisms, respectively. Second, bed sediment samples were obtained from exposed gravel bars with low vegetation cover. The surface sediments were characterized using the pebble count



method (Wolman 1954). We then elaborated cumulative grain-size distributions and derived the ratio between the median surface and subsurface grain-sizes ( $D_{50s}$  and  $D_{50ss}$  respectively) as a measure of bed armoring. We combined the flow metrics, the degree of bed armoring, drainage area (as it mediates the stochastic component of flow variation, sensu Sabo & Post 2008; Sabo *et al.* 2010) and the number of hydraulic infrastructures regulating streamflow in a Principal Components Analysis (Table S3). Replicating the approach of McHugh *et al.* (2010), we used the scores of the first axis of this PCA (Table S3) as an integrative “disturbance index” (hereafter, *DIST*).

We addressed the productivity hypothesis in two complementary ways: by studying ecosystem metabolism (hereafter, *MET*) and by quantifying the availability of basal resources that support the stream food web (hereafter, *RES*). We studied the stream metabolism because this is one of the most integrative measures of river ecosystem functioning, it is meaningful across all types and sizes of streams and rivers, and it indicates energy and material fluxes through ecosystems. Stream ecosystem metabolism rates – gross primary production (GPP) and ecosystem respiration (ER) – can be measured fairly accurately in all types of lotic systems (Tank *et al.* 2010) using the open-channel technique (Odum 1956; Reichert, Uehlinger & Acuña 2009). In particular, ecosystem flux (i.e., the sum of GPP and ER in absolute values) was used as *MET*, as an indicator of the total flux of energy in the ecosystem (see detailed methods and measurements in Figure S3).

To characterize *RES* we mapped the streambed by identifying *in situ* the different patches of biofilm types, macrophytes, and mosses present in each reach. We used a 30 × 30 cm viewer to estimate the relative % cover of each patch every 30 cm, along eleven transverse transects evenly spaced (i.e., every ten meters) in each reach. When present, we also considered coarse

particulate organic matter (CPOM) of terrestrial origin, such as decaying leaves from riparian trees and herbaceous riparian vegetation; fine particulate organic matter (FPOM); and emerged substrata. Samples of each patch were collected at each reach. Biofilms were differentiated by their color, mucilage presence, and the presence of macrocolonies. We randomly selected five stones from the stream bottom for each of the most representative biofilm patches at each reach, and scraped the stones with a knife to fully detach the algal communities (five 2-10 cm<sup>2</sup> replicates/reach). Samples of macrophytes and mosses were collected using a metallic 900 cm<sup>2</sup> frame (3 replicates/reach). CPOM and FPOM were collected using a sediment corer (314 cm<sup>2</sup>, 3 replicates/reach). Samples were frozen (-20°C) and processed to obtain ash free dry weight estimates (AFDW, in g/m<sup>2</sup>). We finally obtained the AFDW of basal resources per reach by scaling up the AFDW of each patch with the percent cover obtained from the streambed mapping and the previous reach size estimates.

Finally, we characterized habitat size (hereafter, *SIZE*) as the wetted cross-sectional perimeter (after McHugh *et al.* 2010). Cross-sectional perimeter was measured in the field at each of the previously-defined eleven transverse transects, and the reach average provided a measure of habitat size that was independent of flow variation (unlike drainage area; Sabo & Post 2008; Sabo *et al.* 2010). Topographic data were obtained through RTK-GPS surveying. A Leica GS15 Real Time Kinematic GPS system was employed, with topographic 3D data quality (position and elevation) being lower than 0.05 m. Field measures were done in spring and summer to consider seasonal variation.

Food-web sampling and sample processing

Potential basal resources included the different biofilm types, macrophytes and bryophytes, benthic CPOM and FPOM, and suspended particulate organic matter (SPOM). We

collected two composite samples per biofilm type for taxonomy (for diatom and for non-diatom algae respectively, preserved in 4 % formaldehyde), and three samples per biofilm type for SIA (frozen to -20 °C in the field), from the same previously selected stones (2-10 cm<sup>2</sup>/sample). Up to 400 diatom valves per slide were identified to species level using Nomarski differential interference contrast at 1000x (Nikon Eclipse 80i, Japan), whereas non-diatom algae were identified after counting 50 random fields per aliquot. Macrophytes and bryophytes (three samples per patch) were collected and divided into two subsamples for species identification and SIA respectively. Subsamples for taxonomic identification were preserved in zip-lock bags at 4°C and were identified upon arrival in the laboratory. CPOM and FPOM were collected using the same methods previously described for basal resources sampling, and SPOM by filtering stream water through precombusted Whatman GF/F filters (three replicates per reach). These samples were frozen (-20 °C) in the field.

We collected 8 Surber replicates (area 625 cm<sup>2</sup>; mesh aperture 250µm) per reach for benthic macroinvertebrates, covering two different riffle areas and one pool at each reach. These samples were preserved in 70 % ethanol and all individuals were sorted from debris in the laboratory. Taxa were identified to the lowest possible taxonomic level (mostly to species level; Diptera to genus/subfamily level; Annelida to subclass level) and counted. A further qualitative sample across all habitats was collected for SIA using a dip-net (20 cm diameter, mesh aperture 500 µm), and sorted in the field. Individuals were kept for 24 h in filtered stream water to allow for gut clearance, and were subsequently frozen (-20 °C).

Finally, aquatic vertebrates were sampled by depletion electrofishing of the entire 100 m survey stretch with a SmithRoot backpack engine (200–350 V, 2–3 A fully rectified triphasic DC). We used stop-nets at both ends of the stretch, and three runs were completed moving

upstream and sweeping from one side of the stream to the other. All fishes and adult reptiles and amphibians were identified, counted and measured (length and body mass). In each reach, up to 3 individuals per species and cohort were euthanized and frozen (-20 °C) in the field for SIA.

Stable isotope analysis

SIA samples were thawed in the laboratory, mollusks were removed from their shells and all three types of samples (basal resources, macroinvertebrates, and vertebrates) were dried at 55 °C until a constant weight was reached. Then, samples were ground using a mortar and pestle. For basal resources, three replicates (one for each sample, 1 - 3 mg each) were loaded into tin capsules. In most cases macroinvertebrate samples consisted of several individuals from the same species and reach (three replicates of  $\approx 500 \mu\text{g}$  each). For larger taxa (e.g., Coleoptera, Heteroptera), each replicate came from a different individual. For vertebrate samples, three replicates of muscle tissue were analyzed for each individual ( $\approx 500 \mu\text{g}$  each). This delivered a total of 819 samples. Carbon (C) and nitrogen (N) content and stable isotopes of C and N were analyzed on a Flash 1112 elemental analyzer connected to a Delta C isotopic ratio mass spectrometer with ConFlo III interfase (Thermo Scientific, Inc.). Stable isotope data were expressed as the relative difference between ratios ( $R$ ) of samples and standards (PeeDee Belemnite limestone for  $\delta^{13}\text{C}$  and atmospheric nitrogen for  $\delta^{15}\text{N}$ ), and expressed in per mille delta notation [i.e.,  $\delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$ ]. Analytical precision from multiple runs was 0.1 ‰ for  $\delta^{13}\text{C}$  and 0.2 ‰ for  $\delta^{15}\text{N}$ .

FCL and dietary proportions

On the one hand, we measured FCL following the maximum trophic position (MTP) convention, as follows. First, we visualized  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  isotopic data and grouped samples based on their signals, resulting in six trophic compartments: POM (CPOM+FPOM+SPOM),

biofilm and algae, macrophytes, primary consumers, secondary consumers, and top predators (consumers were classified after Doadrio 2002; Pleguezuelos, Márquez & Lizana 2002; Tachet *et al.* 2002). Second, we assessed trophic positions of consumers by comparing their  $\delta^{15}\text{N}$  to the mean  $\delta^{15}\text{N}$  of all basal resources in that reach (assuming a 3.4 ‰ fractionation factor, after Post 2002b). FCL was then obtained as the maximum trophic position of the top predator species in a given reach, defined as:

$$\max\{\tau_i | i \in T\} \quad (\text{Equation 1})$$

where  $\tau_i$  is the trophic position of species  $i$  from  $T$ , the set of putative top predators present in that river reach.

On the other hand, dietary proportions were **estimated** using ‘SIAR’, an R package that solves mixing models for isotopic data within a Bayesian framework (Parnell *et al.* 2010). SIAR estimates probability distributions of resource contributions to a primary consumer diet by accounting for variation and uncertainties in the input data. In this study we followed the fractionation factors published in Post (2002b), i.e.,  $1.3 \pm 0.4$  ‰ for  $\delta^{13}\text{C}$  and  $3.4 \pm 1.0$  ‰ for  $\delta^{15}\text{N}$ . Given the potential effects that variability in fractionation factors can have on **stable-isotope mixing models** (Bond & Diamond 2011), we validated that inferences on dietary proportions did not change if we used one of the lowest fractionation factors for  $\delta^{15}\text{N}$  reported in the literature (i.e., 2.54‰; Vanderklift & Ponsard 2003). We also corrected fractionation factors to account for the number of trophic levels between the consumer and the resources (Phillips *et al.* 2014). **Because we had found no visible trace of calcium carbonate on basal resources samples, we did not acidify them.  $\delta^{13}\text{C}$  data showed however that basal resources were slightly enriched relative to primary consumers, with mean  $\delta^{13}\text{C}$  per site ranging from -28.8 to -20.2 ‰ (study-wide mean: -24.6 ‰) for basal resources, and from -33.4 to -27.9 ‰ (study-wide**

mean: -30.8 ‰) for primary consumers. For secondary consumers, mean  $\delta^{13}\text{C}$  per site ranged from -31.8 to -27.4 ‰ (study-wide mean: -29.7 ‰), and for top predators, from -30.5 to -25.2 ‰ (study-wide mean: -28.3 ‰). We ran three different mixing models: (i) one considering the top predators' assemblage as the consumer group, and the 5 lower trophic compartments as potential sources; (ii) one considering the secondary consumers' assemblage as the consumer group, and the 4 immediately-lower trophic compartments as potential sources; and (iii) another one focusing on the most widespread top predator in the study, *Barbus* sp. (following the same layout as for the top predators' assemblage model). All modeled feeding links were ecologically reasonable according to the literature (Doadrio 2002; Pleguezuelos, Márquez & Lizana 2002; Tachet et al. 2002) and to occasional gut content analyses of collected fish (results not shown). The last model, focusing on *Barbus* sp., allowed testing if inferences depended on the level ecological organization being studied (i.e., assemblage vs. one particular species).

Data analysis

First, we qualitatively assessed variation in realized FCL, in the species identity of the predator holding the highest Maximum Trophic Position (MTP), and in the MTP's of each putative top predator, across reaches. We also estimated trophic diversity in each reach by quantifying the isotopic niche space occupied by predators (Layman *et al.* 2007, 2012). To this end we first visualized  $\delta^{15}\text{N}$ -  $\delta^{13}\text{C}$  predators' isotopic data with convex hulls (i.e., the total area of a convex polygon encompassing all species at a site). Then, we fitted a Standard Ellipse Area (SEA) to each food web. Unlike convex hulls, SEA's are robust to variation in sample size. Here we estimated SEA's via Bayesian inference (following Jackson *et al.* 2011) to allow for a more robust comparison among local food webs differing in number of species, samples, and hence variability in the input isotopic data. We made 10,000 posterior draws at each site, and plotted

the distribution of posterior ellipses to test for patterns in estimated trophic diversity across reaches. These analyses were made using ‘SIBER’ (Stable Isotope Bayesian Ellipses in R), a subset of functions of the ‘SIAR’ package (Parnell *et al.* 2010) in R (R Development Core Team 2015) that allows for analysis of data in isotope-space (as opposed to diet-space).

Second, we tested to what extent each putative environmental control explained variation in FCL. To this end we used linear regressions with DIST, MET, RES and SIZE as explanatory variables and FCL as a response variable.

Third, we applied an information-theoretic model-selection approach (Burnham & Anderson 2002) to test the weight of evidence supporting each hypothesized environmental control on the proximate structural mechanisms that may allow variation in FCL (i.e., *additive*, *insertion*, and *omnivory*, Figure 1). We followed a step-wise selection procedure, and models were ranked according to their support, as indicated by Akaike’s Information Criterion corrected for small sample size, AICc. We explored additive mechanisms (Figure 1.i) by testing if any environmental control was significantly related to differences among reaches in top predator composition (i.e., in the identities of the top-predator assemblage), and in top predator structure (i.e., in the relative abundances of the top-predator species). We used the Bray-Curtis coefficient to describe similarities in structure, and the Sorensen coefficient to describe similarities in composition. We addressed insertion mechanisms (Figure 1.ii) similarly, by relating similarities in non-top-predator taxa among these reaches to the set of potential environmental controls. In particular, we performed two additional distance-based linear models; relating putative controls to similarity matrices of invertebrate composition (Sorensen coefficient) and structure (Bray-Curtis coefficient). Finally, in order to test for omnivory mechanisms (Figure 1.iii) we used the dietary proportions obtained from the Bayesian mixing models. In this case, we tested if any



environmental control influenced the diets of the top predators' assemblage, of the secondary consumers' assemblage, and of *Barbus* (thus adding three additional models). If a particular structural mechanism responded significantly to a FCL environmental control, it could potentially mediate environment-FCL relationships.

Last, because variation in predator body size could also influence FCL (i.e., bigger predators can eat bigger prey), we tested if *Barbus* size distributions changed across reaches, and if *Barbus* body size (fork length) correlated with its trophic position.

**Results**

Food-web overview

Macrophytes and bryophytes largely dominated basal resources biomass, with 7 macrophyte and 2 bryophyte species that combined accounted for 75.9 % ( $\pm$  11.9 SE) of the total resource biomass. Biofilm and algae accounted in turn for 5.5 % ( $\pm$  2.3 SE), with 118 taxa of diatoms and 52 of non-diatom algae and cyanobacteria. A total of 89 macroinvertebrate taxa were detected, of which 41 were primary consumers and 48 were predators. We also captured 10 species of aquatic vertebrates, including 6 putative top predators: the viperine water snake *Natrix maura*, and the fishes *Anguilla anguilla*, *Barbus* sp. [cf. *graellsii* x *haasi*], *Gobius lozanoi*, *Salmo trutta*, and *Squalius laietanus*.

Reaches hosted 1 - 4 top predator species and FCL ranged from 1.9 to 4.0 (Table 2, Figure 3). *Natrix maura* and *Barbus* sp. presented a very high MTP range ( $>1$  trophic level), *Barbus* sp. and *Anguilla anguilla* were the species that most often set the FCL (in 3 reaches each), and *Barbus* was the top predator most commonly found, present everywhere except in the fishless reach M1 (Table 2). The biggest leap in FCL occurred, in both Siurana and Montsant



321 rivers, between the most upstream reach (unregulated) and the reach just downstream of the first  
 322 dam: between reaches M1 and M2 the food chain lengthened 1.5 trophic levels, and between  
 323 reaches S1 and S2, 0.9 trophic levels (Table 2, Figure 3). Interestingly, FCL was not positively  
 324 correlated with trophic diversity (Spearman's  $Rho = -0.06$ ; Figure 3). This indicates that  
 325 downstream increases in FCL were not driven by an increase in the diversity of trophic pathways  
 326 used by predators. Instead, trophic diversity decreased non-significantly between reaches M1 and  
 327 M2; and significantly (as indicated by the non-overlap of 95% credible intervals) between  
 328 reaches S1 and S2, almost halving (Figure 3).

### 329 FCL environmental controls

330 Reaches differed in disturbance (DIST), ecosystem metabolism (MET), standing basal  
 331 resources (RES), and wetted perimeter (SIZE), but showed low covariation among these  
 332 variables (Figure S4). Hydrological disturbance (DIST) was the most evident FCL environmental  
 333 control (Figure 4). Lowest hydrological disturbance (DIST) values represent relatively more  
 334 regulated reaches, larger in drainage area and less affected by high and low flows (Table S3).  
 335 Thus, as expected, regulation increased FCL.

### 336 FCL proximate structural mechanisms

337 The set of distance-based linear models testing the influence of environmental controls on  
 338 the three proximate structural mechanisms of FCL provided complementary information (Table  
 339 3). Out of the seven models run (two testing for additive, two testing for insertion, and three  
 340 testing for omnivory mechanisms; Table 3), four showed statistically-significant associations, of  
 341 which three selected DIST (either alone or combined with another control) as the best predictor.  
 342 DIST had no effect on additive mechanisms, and had a significant but small effect in insertion

mechanisms (Table 3). In contrast, omnivory mechanisms of the top predators' assemblage were largely explained by DIST; and omnivory mechanisms of *Barbus*, by a combination of DIST and SIZE (Table 3). When comparing dietary proportions across the study reaches, notable patterns were observed for top predators in general and for *Barbus* in particular (Figure 5): the most stable reaches (i.e., those exhibiting longer food chains) hosted top predators that fed more on secondary consumers and less on basal resources (POM, biofilm and algae), whereas the opposite was true in the relatively more disturbed, short-FCL reaches (Figure 5b). Similarly, primary and secondary consumers contributed most to *Barbus* diets in the low bookend of the disturbance gradient (Figure 5c). We found no apparent relationship between *Barbus* individual trophic position and body size, despite the wide range of trophic positions (2.2-3.6) and sizes considered (55-320 mm; Figure 6). Therefore, FCL was not a function of bigger *Barbus* in some reaches.

**Discussion**

Regulation dampened discharge variation by muting high and low flows, and this unnatural hydrologic stability controlled food chain length (FCL) in a Mediterranean river network. Omnivory mechanisms of top predators allowed food webs to shift from short- to long-FCL configurations, with hydrologic stability increasing intraguild predation (and hence FCL). This finding implies that even if no species colonize or become locally-extirpated as a consequence of flow regulation, food-web structure may change via shifts in the dominant energy pathways. These results may help better understanding the impacts of flow regulation on structure and function of highly-seasonal streams. Free-flowing streams in Mediterranean-climate regions typically exhibit strong intra- and inter-annual variability (Gasith & Resh 1999; Bonada & Resh 2013), hosting biotas with adaptive suites of traits (Bonada, Doledec & Statzner

2007). Dams in Mediterranean rivers lower biodiversity (Clavero, Blanco-Garrido & Prenda 2004) and here we provide evidence that they also impact riverine food-web structure (as suggested by Power *et al.* 1996).

#### Flow regulation and FCL controls

The observed FCL average ( $3.2 \pm 0.2$ ; mean  $\pm$  SE) was slightly lower than the global FCL average reported for streams (i.e., 3.5; Vander Zanden *et al.* 2007). FCL variation across fish-bearing sites in our study (1.7 trophic levels) was higher than the variation between the fishless site and the lowest-FCL fish-bearing site (0.4 trophic levels). Therefore, although dispersal barriers probably prevented fish being part of the local food web in a particular reach (sensu Power & Dietrich 2002), hydrological disturbance shortened food chains consistently, regardless of which group or species held the Maximum Trophic Position in each reach.

We confirmed the prediction that flow variation would be a major determinant of FCL in our basin, with productivity (tested through resource availability and ecosystem metabolism) and habitat size playing secondary roles. The disturbance (or dynamical stability) hypothesis has been supported in a variety of freshwater ecosystems, including artificial phytotelmata (Jenkins, Kitching & Pimm 1992) and ponds (Schneider 1997; Schriever & Williams 2013). However, in running waters results have been mixed: some studies did not find relevant effects of bed disturbance (Townsend *et al.* 1998) or hydrological disturbance on FCL (Walters & Post 2008), whereas others reported negative effects (McHugh *et al.* 2010; Sabo *et al.* 2010). Differences in methods could cause these inconsistent results, as could the difficulty of disentangling confounding variables (e.g., across different streams or catchments) in large-scale field studies. Our results agree with theory, and with a large-scale field study that reported FCL decreasing with hydrologic variability across 36 American rivers, with drainage area mediating flow

variation (Sabo *et al.* 2010). In our study regulation scaled flow variation, and equivalent patterns to those observed by Sabo *et al.* (2010) were found in a single river basin.

The effect of regulation on food-web structure via flow variation may be paramount, but our results cannot exclude that regulation may also affect food-web structure via the productivity and the ecosystem size hypotheses, or via the interaction between these two (coined as the productive-space hypothesis; Schoener 1989). In this vein, dams increase the river's capacity to store and process materials and energy in downstream reaches (Aristi *et al.* 2014; von Schiller *et al.* 2015), and energy supply has been identified as a significant influence of food-web structure in streams (Thompson & Townsend 2005). Because the effects of productivity and resource availability only seem to be apparent when very low bookends of these controls are considered (Post 2002a), the productivity hypothesis may come into play only if regulation fundamentally expands the range of resource availability and/or GPP and ER. This did not seem to be the case in this study (Figure S3), and our negative result supports previous tests of the effects of GPP or standing crop biomass of resources on riverine FCL (McHugh *et al.* 2010; Sabo *et al.* 2010). We also acknowledge the short gradient in habitat size considered in our study, which may contribute to explain why this control did not influence FCL. Habitat size effects have been corroborated in well-delimited ecosystems such as lakes (Vander Zanden *et al.* 1999; Post *et al.* 2000) and ponds (Doi *et al.* 2009), whereas studies in running waters have been less conclusive (but see Thompson & Townsend 2005; McHugh *et al.* 2010). A positive association between habitat size and FCL would have probably emerged by means of species-area relationships (Holt *et al.* 1999) if we had expanded the size gradient by including higher-order reaches. However, such expansion (and hence the habitat size - FCL association) would have not reflected the impact of flow regulation by dams, which was the aim of our study.

### Omnivory mechanisms link flow regulation to FCL

Flow variation also controlled omnivory mechanisms, with top predators in unregulated reaches feeding relatively more on several trophic levels. This pattern matches our prediction and provides empirical evidence of omnivory mechanisms connecting flow variation to FCL in streams. Although this link had been previously proposed (Parker & Huryn 2006; Post & Takimoto 2007; McHugh *et al.* 2010; Takimoto & Post 2013), it had not been yet tested, to our knowledge, in the context of flow regulation.

A previous study had shown that hydrologic disturbance may shorten arctic stream food chains by limiting the contribution of secondary consumers to predator fish diets (Parker & Huryn 2006). Similarly, *Lepidodactylus* geckos had higher proportions of predators in their stomach contents when found on high- than on low-productivity islets, with productivity and predator size also influencing FCL across the set of islets (Young *et al.* 2013). Because in nature prey tend to be smaller than their predators (Woodward *et al.* 2005), variation in predator body size could be actually driving omnivory and hence FCL patterns (Hoeinghaus *et al.* 2008). However, when we tested this hypothesis we found no apparent relationship between these variables. Therefore, even if flow variation regimes could partly explain top predators' size distributions across our reaches (as in Jellyman, McHugh & McIntosh 2014), changes in their trophic positions likely reflected "forced" diet shifts due to disturbance-driven scarcity of invertebrates, rather than ontogenetic diet shifts.

### Concluding remarks

Changes in FCL may present relevant implications in terms of pollutant bioaccumulation by animals (Cabana & Rasmussen 1994), nutrient recycling (McIntyre *et al.* 2007), and carbon exchange between freshwater ecosystems and the atmosphere (Schindler *et al.* 1997). It is

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3 435 therefore important to understand the links between increasing hydrologic alteration in rivers and  
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5 436 FCL. It had been previously suggested that the ultimate effects of river regulation on FCL may  
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8 437 depend on whether regulation increases the proportion of “protected” consumers (e.g., predator-  
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10 438 resistant grazers), which may subsequently disrupt energy flow to higher trophic levels and thus  
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12 439 reduce the abundance of intraguild-predators (Wootton *et al.* 1996). In our study we found no  
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14 440 evidence of “protected” consumers subsuming the effects of flow regulation (confirmed by  
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16 441 occasional gut content analyses of collected fish). Instead, effects rippled through the food web  
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18 442 via omnivory mechanisms—ultimately affecting FCL. Our design tested for flow-regulation  
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20 443 effects in a semi-controlled manner (i.e., in a single small basin and pool of organisms), but this  
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22 444 was at the expense of sacrificing replication. Further research should ask whether food chain  
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24 445 lengthening is a general or a contingent consequence of flow regulation; whether these effects  
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26 446 are consistent intra- and inter-annually; and whether omnivory mechanisms are universal or  
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28 447 replaced by additions/insertions when larger spatial scales (and hence potential predator  
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30 448 extirpations due to riverine habitat fragmentation) are considered. Given the multitude of dams  
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32 449 already present or planned across the globe, it is crucial that we advance our understanding on  
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34 450 their impacts at the higher levels of biological organization.  
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**Supporting Information**

**Table S1.** Summary of the physico-chemical variables considered.

**Table S2.** Permutation-based models combining data on physico-chemical variables and biota.

**Table S3.** Details of the Principal Component Analysis (PCA) combining hydrological and geomorphological data.

**Figure S1.** Principal Components Analysis (PCA) ordering the 9 studied reaches according to their physico-chemical characteristics.

**Figure S2.** Observed (Q obs) versus simulated flow (Q sim) at the Siurana reservoir.

**Figure S3.** Ecosystem metabolism (MET) across the studied reaches.

**Figure S4.** Scatter plots showing the correlations among the hypothesized environmental controls of FCL (*DIST*, *MET*, *RES*, *SIZE*).

## 640 Tables

641 **Table 1.** Site characteristics and data used to compute the multivariate stability index (DIST).

642 Drainage area and hydraulic infrastructure control the stochastic component of flow variation

643 (after Sabo *et al.* 2010). The number of low- and high-flow days was obtained after Richer *et al.*

644 (1996), using mean daily flow series from 1/1/1998 to 30/06/2012 (see text for details). The

645 degree of bed armoring is the ratio between the median surface ( $D_{50s}$ ) and the median subsurface

646 ( $D_{50ss}$ ) material (Bunte & Abt 2001) and indicates absence of sediment supply from upstream.

647 M = Montsant river; S = Siurana river; CON = confluence of Montsant and Siurana rivers.

Reach	UTM (x, y)	Drainage area (km <sup>2</sup> )	Regulation	Low flows	High flows	Bed armoring
M1	824850, 4582650	40.66	-	50	56	3.15
M2	816050, 4578550	97.58	Margalef reservoir	26	22	4.92
M3	814850, 4577650	113.1	Margalef reservoir	35	22	4.03
M4	810850, 4573050	141.4	Margalef reservoir	24	28	2.98
S1	830350, 4574750	35.64	-	68	56	5.08
S2	827750, 4573750	60.96	Siurana reservoir	2	13	8.02
S3	826050, 4572150	88.15	Siurana reservoir	2	30	4.98
S4	818050, 4569050	140.2	Siurana reservoir, Riudecanyes weir and bypass	18	32	4.43
CON	814150, 4564150	421.6	Margalef reservoir, Vilella reservoir, Siurana reservoir, Riudecanyes weir and bypass	13	40	2.56

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**Table 2.** Maximum trophic position (MTP) of the putative top predator species in each reach.

The value of the species holding the absolute MTP in each reach (i.e., the one setting FCL) is shown in italics.  $\Delta_{\text{MTP}}$  = MTP range across reaches. M = Montsant river; S = Siurana river; CON = confluence of Montsant and Siurana rivers.

Top predator	M1	M2	M3	M4	S1	S2	S3	S4	CON	$\Delta_{\text{MTP}}$
<i>Natrix maura</i> (reptile)	1.93			3.14		3.26		3.57	3.48	1.64
<i>Anguilla anguilla</i> (fish)		3.43	3.47						4.00	0.57
<i>Barbus</i> sp. (fish)		3.31	3.35	3.38	2.34	3.23	3.43	3.49	3.64	1.30
<i>Gobius lozanoi</i> (fish)		3.17	3.05	3.15						0.12
<i>Salmo trutta</i> (fish)		3.36								-
<i>Squalius laietanus</i> (fish)					2.15			3.64	3.37	1.49

**Table 3.** Set of distance-based linear models testing the influence of environmental controls on the proximate structural mechanisms (additive, insertion, and omnivory mechanisms) of FCL variation (*DIST* = disturbance, *MET* = ecosystem metabolism, *RES* = basal resource availability, *SIZE* = wetted perimeter; see text for details). A step-wise selection procedure based on 9,999 permutations allowed identifying the environmental controls (Var.) that minimized AICc for each of the 7 models. The test statistic (Pseudo-F) and P-value of the selected controls are shown for each best model, together with the marginal percentage of explained variance after entering the variable (Prop.) and the corresponding degrees of freedom (df) of the model. In all cases except for *Barbus* sp. dietary proportions, the best model involved only one control. Statistically-significant control-mechanism associations are shown in bold.

Test	Response matrix	Var	df	AIC <sub>c</sub>	SS (trace)	Pseudo-F	P	Prop.
Additive mechanisms	<b>Top predators composition</b>	<b>RES</b>	<b>7</b>	<b>65.58</b>	<b>5188</b>	<b>5.377</b>	<b>0.036</b>	<b>0.434</b>
	Top predators structure	RES	7	71.57	3830	2.041	0.065	0.226
Insertion mechanisms	Invertebrate composition	MET	7	67.78	2050	1.664	0.097	0.192
	<b>Invertebrate structure</b>	<b>DIST</b>	<b>7</b>	<b>70.85</b>	<b>2966</b>	<b>1.713</b>	<b>0.044</b>	<b>0.197</b>
Omnivory mechanisms	<b>Top predators dietary proportions</b>	<b>DIST</b>	<b>7</b>	<b>52.31</b>	<b>1898</b>	<b>8.600</b>	<b>0.002</b>	<b>0.551</b>
	Secondary consumers dietary proportions	RES	7	51.35	366.1	1.846	0.213	0.209
	<b><i>Barbus</i> sp. dietary proportions</b>	<b>SIZE</b>	<b>6</b>	<b>46.05</b>	<b>823.9</b>	<b>4.352</b>	<b>0.048</b>	<b>0.420</b>
		<b>DIST</b>	<b>5</b>	<b>43.02</b>	<b>749.6</b>	<b>9.703</b>	<b>0.024</b>	<b>0.382</b>

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**Figure legends**

**Figure 1.** Schematization of the proximate structural mechanisms that allow FCL variation (adapted from Post & Takimoto 2007). Stones with green algae represent basal resources, Orthocladiinae non-biting midges represent primary consumers, *Isoperla* stoneflies represent secondary consumers, and Catalanian barbels *Barbus haasi* represent top predators. The three mechanisms may explain transitions between shorter (~3-3.25) and longer (~3.75-4) food-chain configurations (numbers represent trophic positions). For **(i) additive** and **(ii) insertion** mechanisms, constant energy fluxes are assumed between short- and long-FCL food web configurations (connectance food webs). In contrast, **(iii) omnivory** mechanisms are represented in energy-flow food webs, where relatively thicker lines represent stronger fluxes. *Omnivory* mechanisms can be exerted by top predators **(iii.a)** or by secondary consumers **(iii.b)**.

**Figure 2.** Study site map showing the drainage area, the main hydraulic infrastructures that regulate streamflow (Margalef, Siurana and Vilella Baixa reservoirs, Riudecanyes weir and bypass), and the location of the 9 studied reaches. M = Montsant river; S = Siurana river; CON = confluence of Montsant and Siurana rivers. See details in Table 1.

**Figure 3.** Variation in food-chain length and in trophic diversity (bivariate  $\delta^{13}\text{C}$  -  $\delta^{15}\text{N}$  isotopic space) across reaches. Trophic diversity was estimated using Bayesian Standard Ellipse Areas (SEA), with the ‘siber.ellipses’ function of the ‘SIAR’ R-package (Parnell *et al.* 2010). 10,000 posterior draws were made in each case, and the corresponding credible intervals are shown for each local food web. Only predators were included ( $N = 462$ ).

**Figure 4.** Relationships between the hypothesized environmental controls and FCL ( $DIST$  = disturbance,  $MET$  = ecosystem metabolism,  $RES$  = basal resource availability,  $SIZE$  = habitat



size; see text for details).  $r^2$  and  $P$ -values of the linear regressions between each control (explanatory variables) and FCL (response variable) are shown. *DIST* was the only significant regression ( $P < 0.05$ ) and it is indicated by a solid line.

**Figure 5.** Effects of the main environmental control on *omnivory*. The dietary proportions of (a) the secondary consumers' assemblage, (b) the top predators' assemblage, and (c) *Barbus*, are represented across the hydrological disturbance gradient (*DIST*). Each color represents a different trophic compartment being consumed: orange triangles = POM; green diamonds = biofilm + green algae; blue diamonds = macrophytes; grey circles = primary consumers; black circles = secondary consumers (only considered in b and c).  $r^2$  and  $P$ -values of the linear regressions are shown, and significant regressions ( $P < 0.05$ ) are indicated by solid lines.

**Figure 6.** (a) Relationship between *Barbus* sp. individual fork length and its individual trophic position. The  $r^2$  and  $P$ -value of the linear regression between these variables are shown. (b) Size distribution of this taxon across the studied reaches. Sizes differed significantly overall (GLM,  $F_{7,304} = 54.202$ ,  $P < 0.001$ ). Letters show which reaches share a similar size distribution (as identified by Scheffé post-hoc tests).

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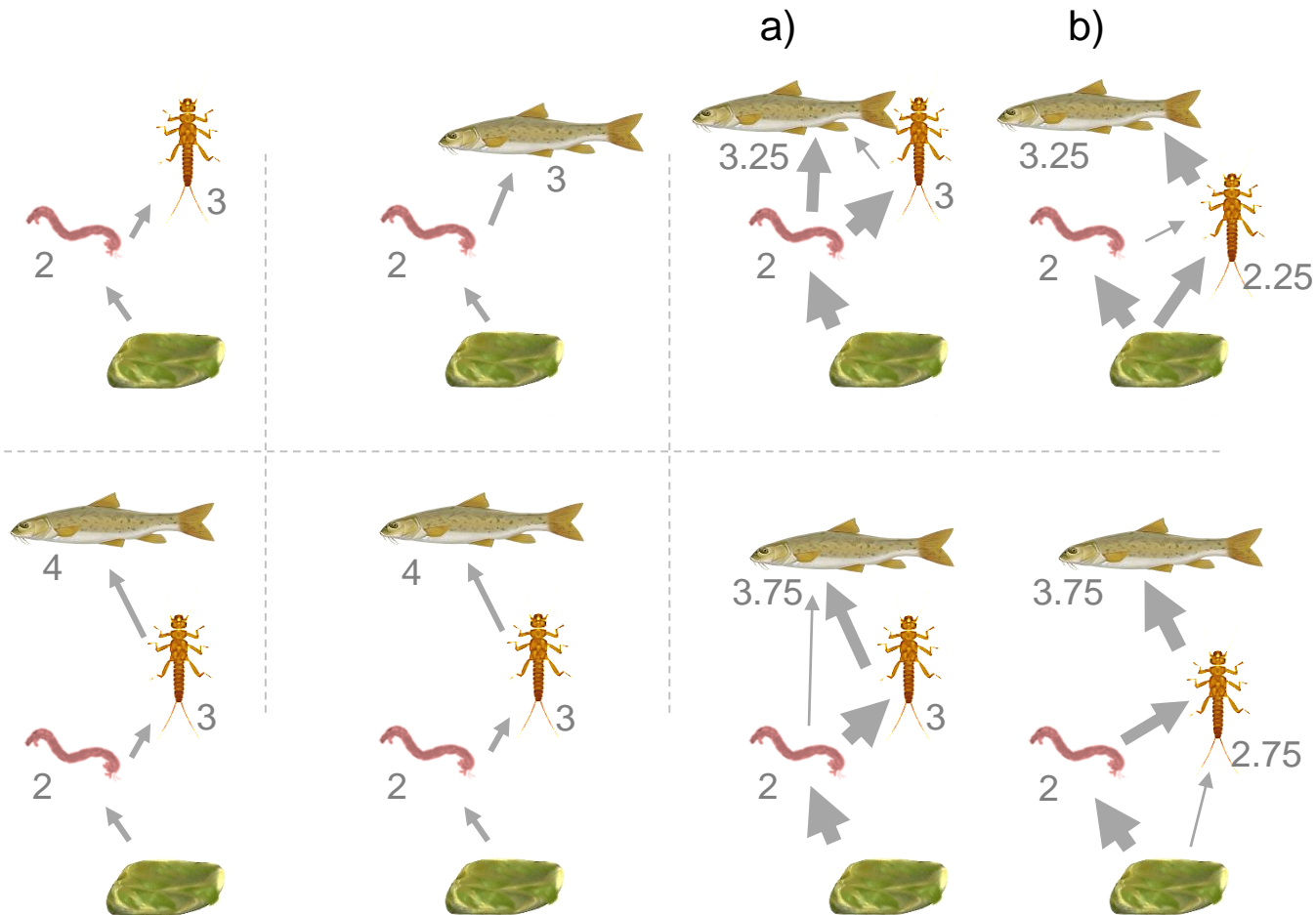
# Proximate structural mechanisms for variation in FCL

(i) additive                      (ii) insertion                      (iii) omnivory

Food web configuration

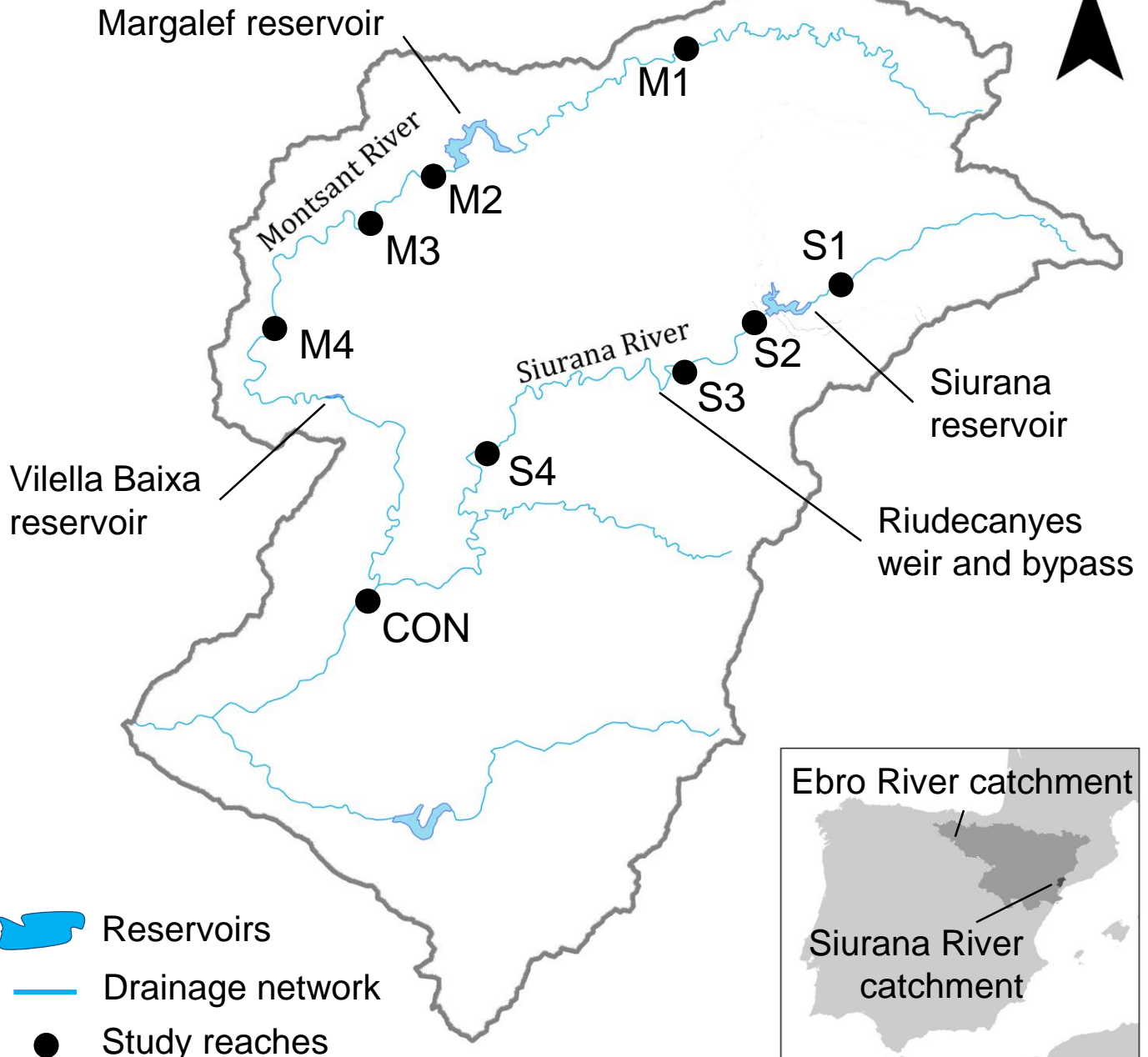
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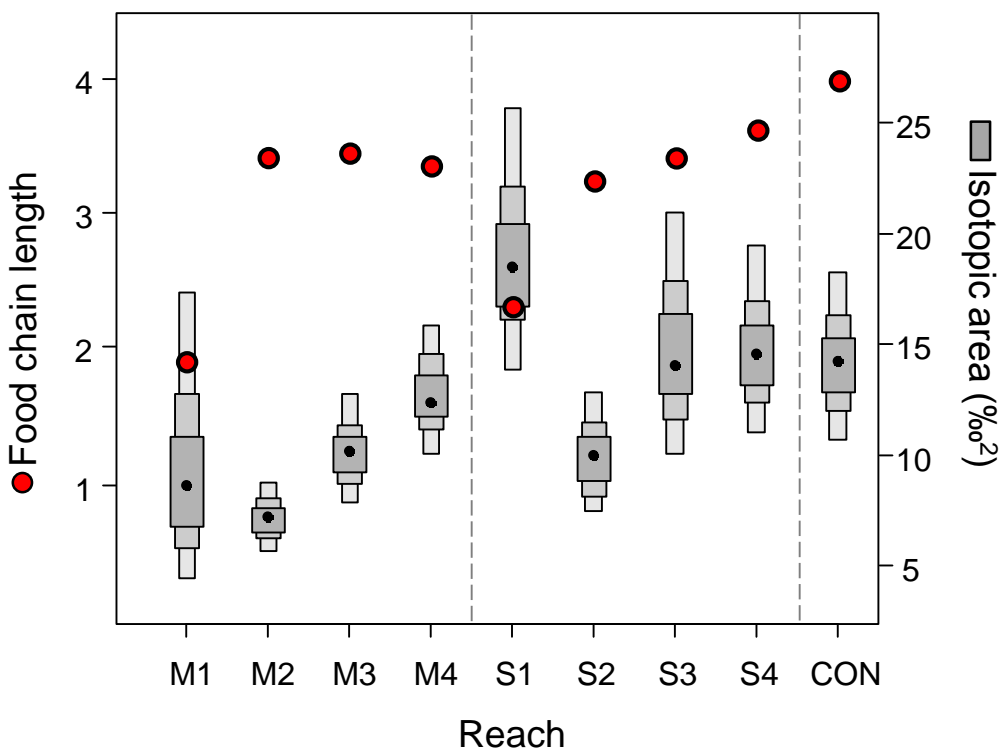
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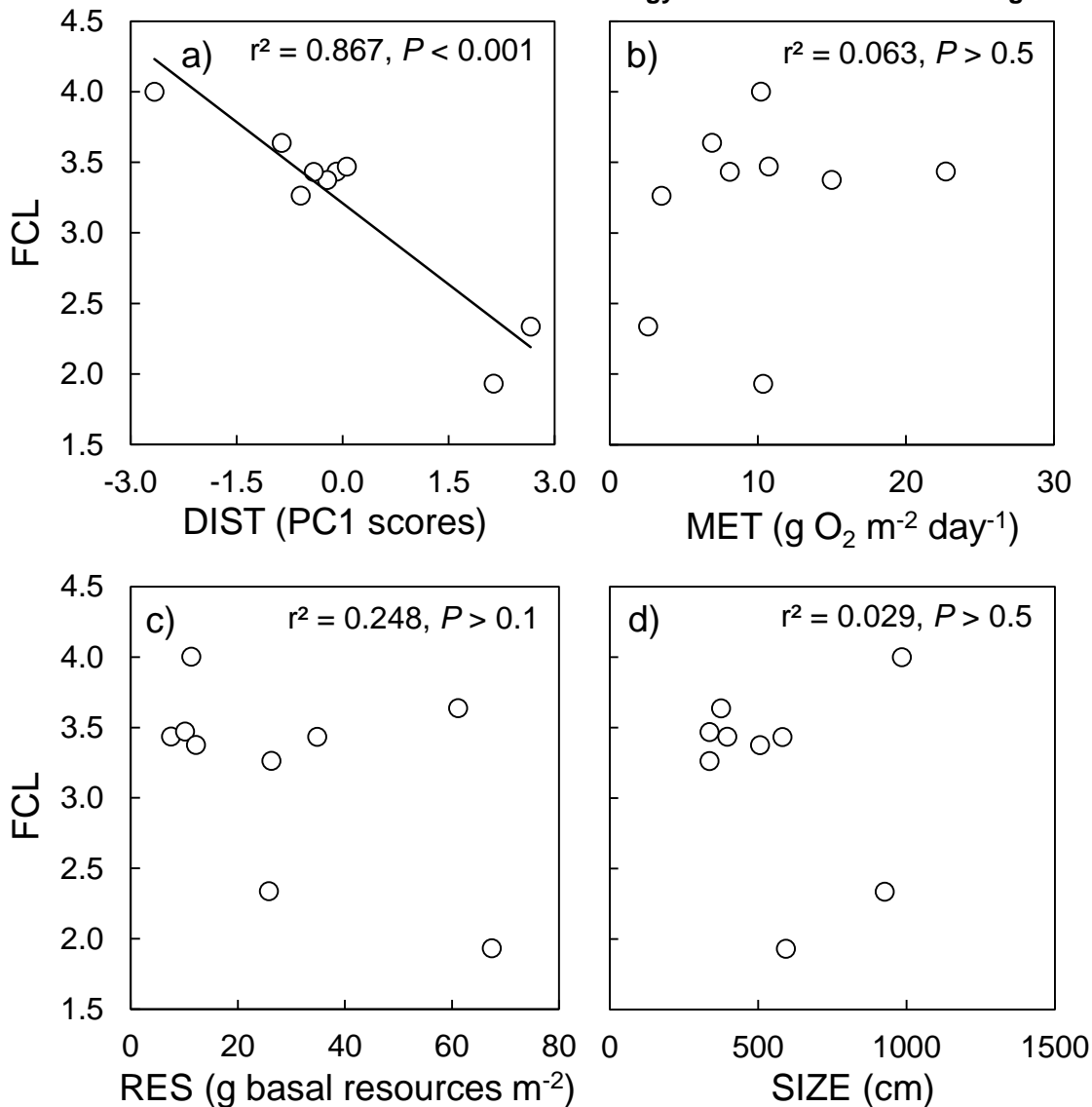


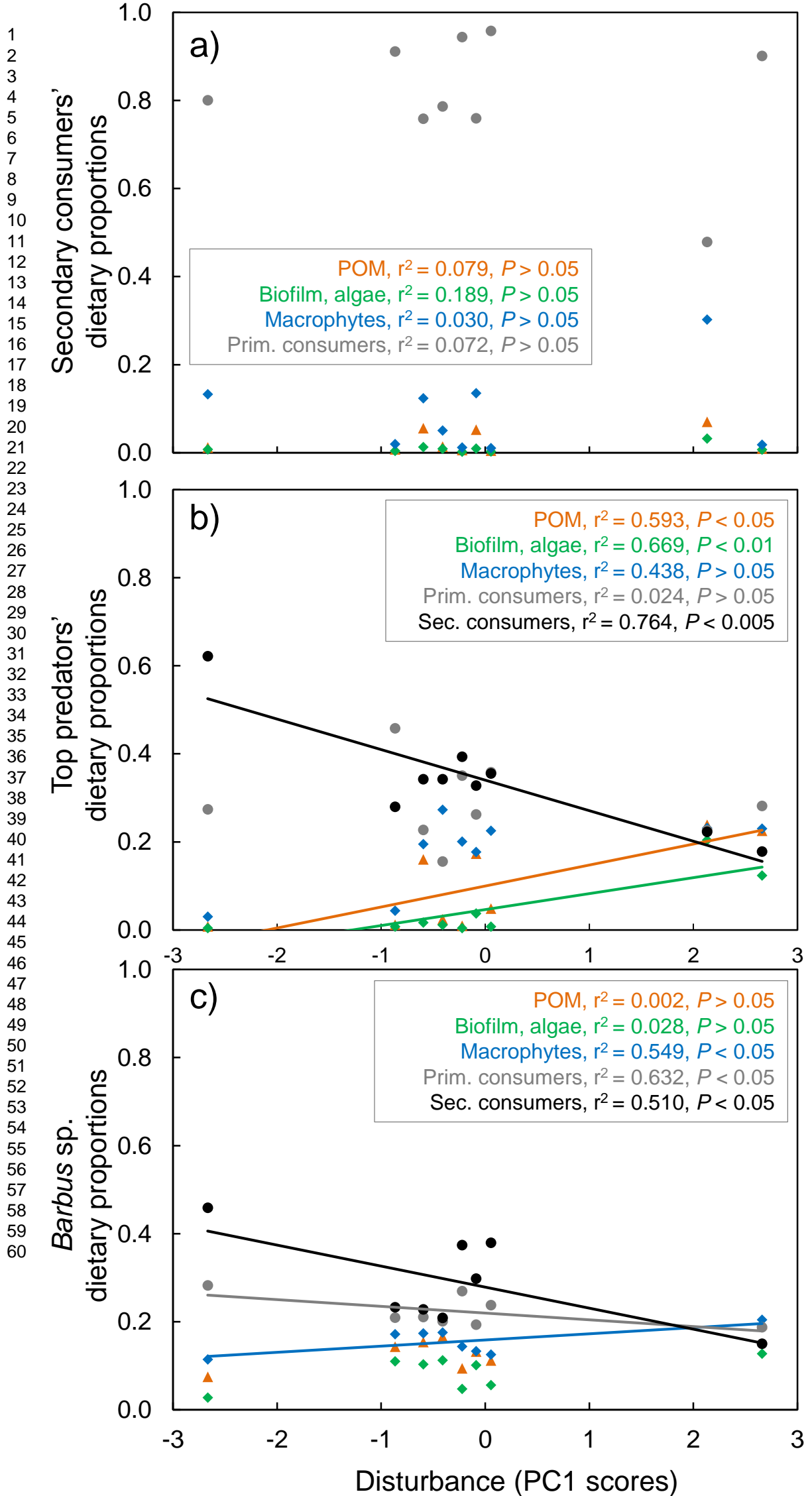
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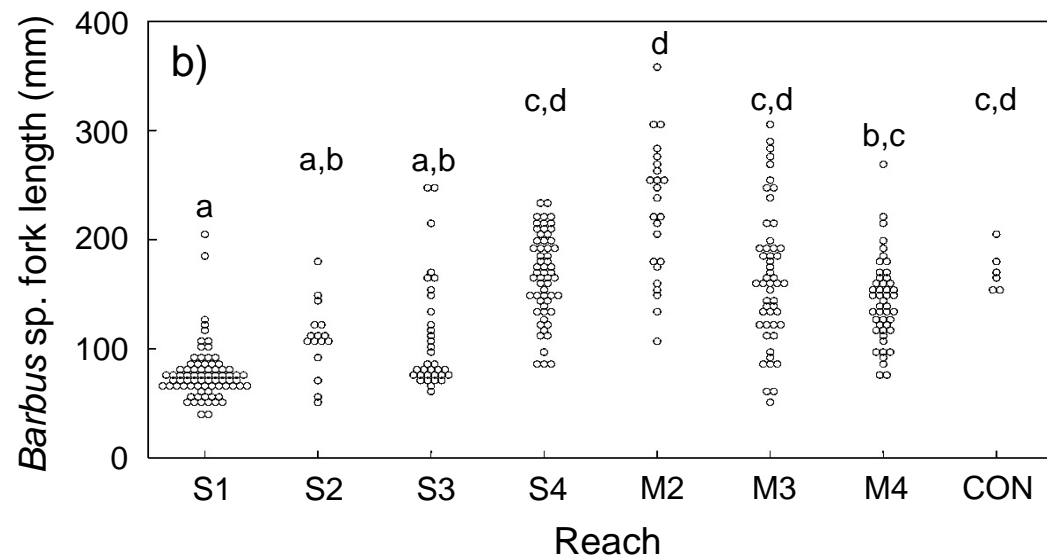
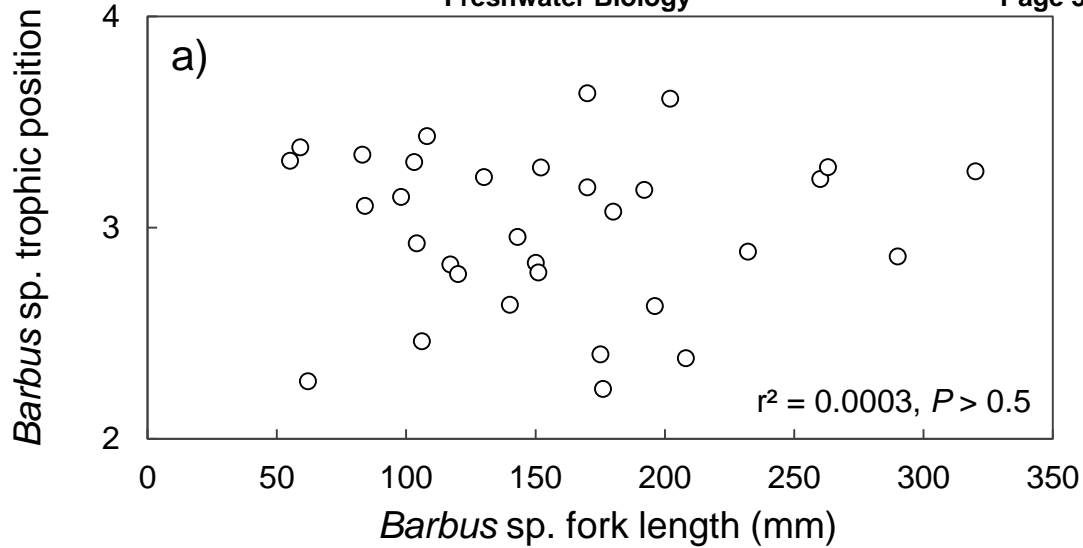
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Supporting Information

Flow regulation increases food chain length through omnivory mechanisms in a Mediterranean river network

Albert Ruhí, Isabel Muñoz, Elisabet Tornés, Ramon J. Batalla, Damià Vericat, Lydia Ponsatí, Vicenç Acuña, Daniel von Schiller, Rafael Marcé, Gianbattista Bussi, Félix Francés & Sergi Sabater

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**Table S1. Summary of the physico-chemical variables considered.** Water temperature (Temp), dissolved oxygen ( $O_2$ ) and conductivity (Cond) were determined in situ using field meters (Hach, Loveland, USA). Water samples for nutrients were collected in triplicate from each reach, at approximately 10 cm below the water surface. These samples were filtered in situ through combusted and pre-weighed glass fiber filters (Whatman, UK) using portable filtration devices connected to a vacuum pressure pump (Millipore, USA). Samples were placed in rinsed polyethylene bottles and frozen ( $-20^\circ\text{C}$ ) in the field until analysis. Total dissolved phosphorus (TDP) was measured as the concentration of phosphate ( $\text{PO}_4^{3-}$ ) after acid digestion in a Selecta Presoclave-II 30L autoclave (JP Selecta, Spain). The concentrations of ammonium ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ) and nitrite ( $\text{NO}_2^-$ ) were determined colorimetrically using an Alliance-AMS Smartchem 140 (AMS, France). The concentrations of total dissolved nitrogen (TDN) and dissolved organic carbon (DOC) were measured on a Shimadzu TOC-V CSH coupled to a TNM-1 module (Shimadzu Corporation, Japan). Values are 3-replicate averages. M = Montsant river; S = Siurana river; CON = confluence of Montsant and Siurana rivers.

Reach	M1	M2	M3	M4	S1	S2	S3	S4	CON
Temp ( $^\circ\text{C}$ )	11.9	13.7	13.4	13.1	19.3	11.2	15.1	20.7	19.5
$O_2$ ( $\text{mg L}^{-1}$ )	9.6	12.1	10.6	7.8	9.1	11.4	9.0	6.2	11.0
Cond ( $\mu\text{S cm}^{-1}$ )	365.7	412.0	432.0	485.0	691.7	581.0	924.0	711.0	547.0
$\text{PO}_4^{3-}$ ( $\text{mg L}^{-1}$ )	0.013	0.008	0.008	0.005	0.008	0.008	0.026	0.007	0.006
TDP ( $\text{mg L}^{-1}$ )	0.030	0.013	0.010	0.016	0.012	0.013	0.043	0.017	0.022
$\text{NH}_4^+$ ( $\text{mg L}^{-1}$ )	0.005	0.001	0.001	0.001	0.003	0.015	0.009	0.003	0.001
$\text{NO}_3^-$ ( $\text{mg L}^{-1}$ )	0.002	0.533	0.377	0.028	0.054	0.069	0.440	0.002	0.015
$\text{NO}_2^-$ ( $\text{mg L}^{-1}$ )	0.003	0.006	0.006	0.005	0.003	0.008	0.006	0.003	0.005
TDN ( $\text{mg L}^{-1}$ )	0.158	0.686	0.442	0.177	0.221	0.300	0.455	0.170	0.165
DOC ( $\text{mg L}^{-1}$ )	1.747	3.030	2.364	1.687	1.256	2.545	1.625	1.687	1.518

**Table S2. Permutation-based models combining data on physico-chemical variables and biota. A)**

Results of the permutational MANOVA (PERMANOVA) employed to explore physico-chemical variation among upstream, downstream, and confluence sections, and within the downstream section. In order to account for the spatially-autocorrelated structure of the study, *reach* factor (random, 9 levels: each reach) was nested within *section* factor (fixed, 5 levels: M1, M2-M3-M4, S1, S2-S3-S4, CON). Physico-chemical conditions did not significantly differ between sections, despite variation across the reaches located downstream of the main reservoirs. **B)** Results of the RELATE and BIO-ENV routines combining the environmental matrix with the algae, invertebrate and vertebrate community matrices. The RELATE procedure (*Rho* and *Sign* columns) is a Mantel-type test that contrasts the null hypothesis of no significant link between two similarity matrices by permuting the sample labels from one of the similarity matrices and recalculating the matches (the rank correlation, *Rho*) many times (999 permutations; *Sign* = significance level of sample statistic expressed as a percentage) (Clarke & Gorley 2006). Subsequently, the BIO-ENV routine (column *Variables selected*) allowed identifying the subset of variables that maximize the rank correlation (Clarke & Gorley 2006). Note that physico-chemical variation did not significantly influence algae, invertebrate, or vertebrate community composition (*Sign* > 5% in all 3 cases). Furthermore, the variables maximizing the rank correlation presented very limited predictive power (*Rho* < 0.35 in all 3 cases). All data were log<sub>10</sub>(X+1) transformed. Euclidean distances were used for the physico-chemical matrix, and Bray-Curtis coefficient for the algae, invertebrate and vertebrate matrices.

A)	df	SS	MS	Pseudo-F	P(perm)
Section	4	3.341	0.835	1.229	0.333
Reach(Section)	4	2.718	0.680	83.57	0.001
B)	Rho	Sign	Variables selected		
Algae community	0.317	66%	NO <sub>2</sub> <sup>-</sup>		
Invertebrate community	0.347	45%	Cond		
Vertebrate community	0.315	47%	TDN, Temp, Cond		

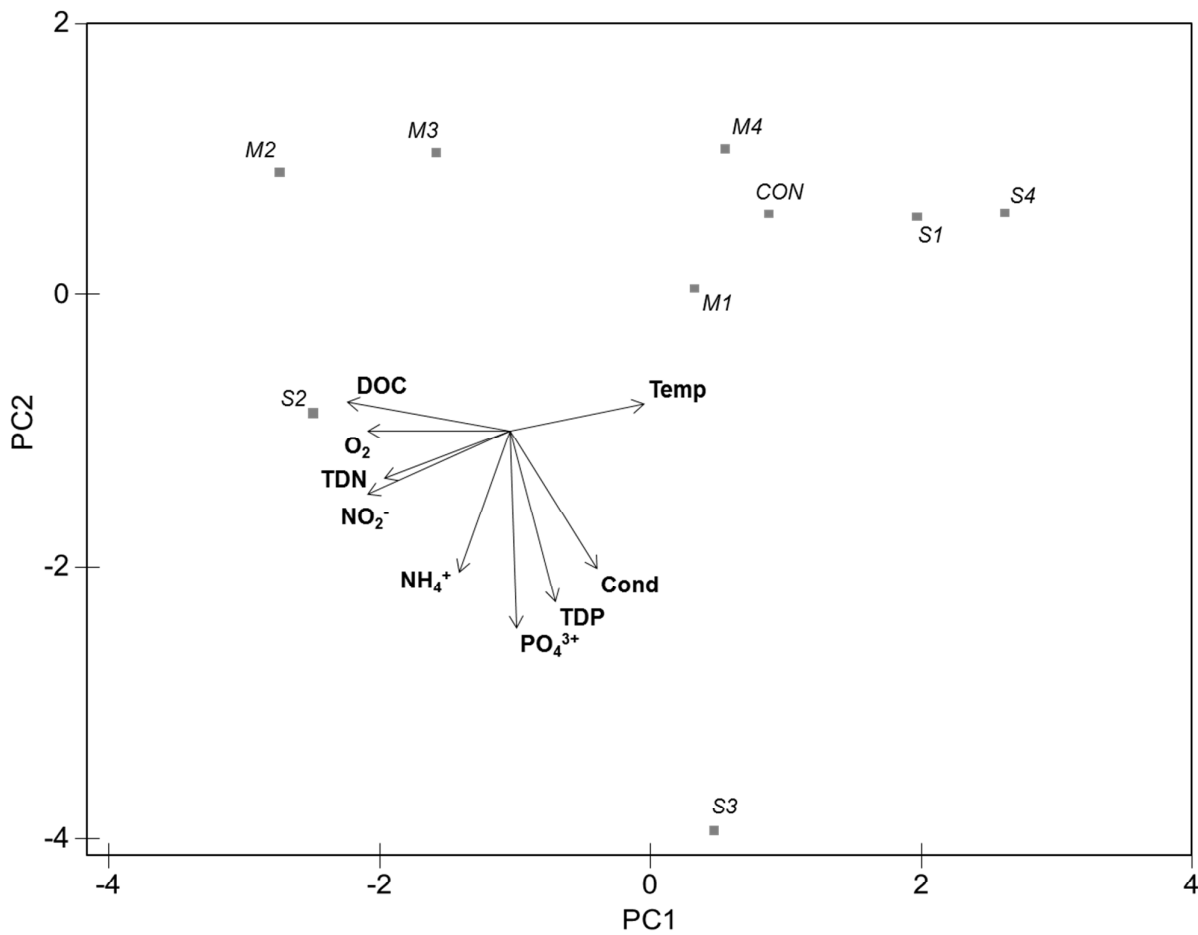
**Table S3. Details of the Principal Component Analysis (PCA) combining hydrological and geomorphological data.** PC1 was selected as our multivariate index of stability (*DIST*).

Principal Component Analysis (PCA):

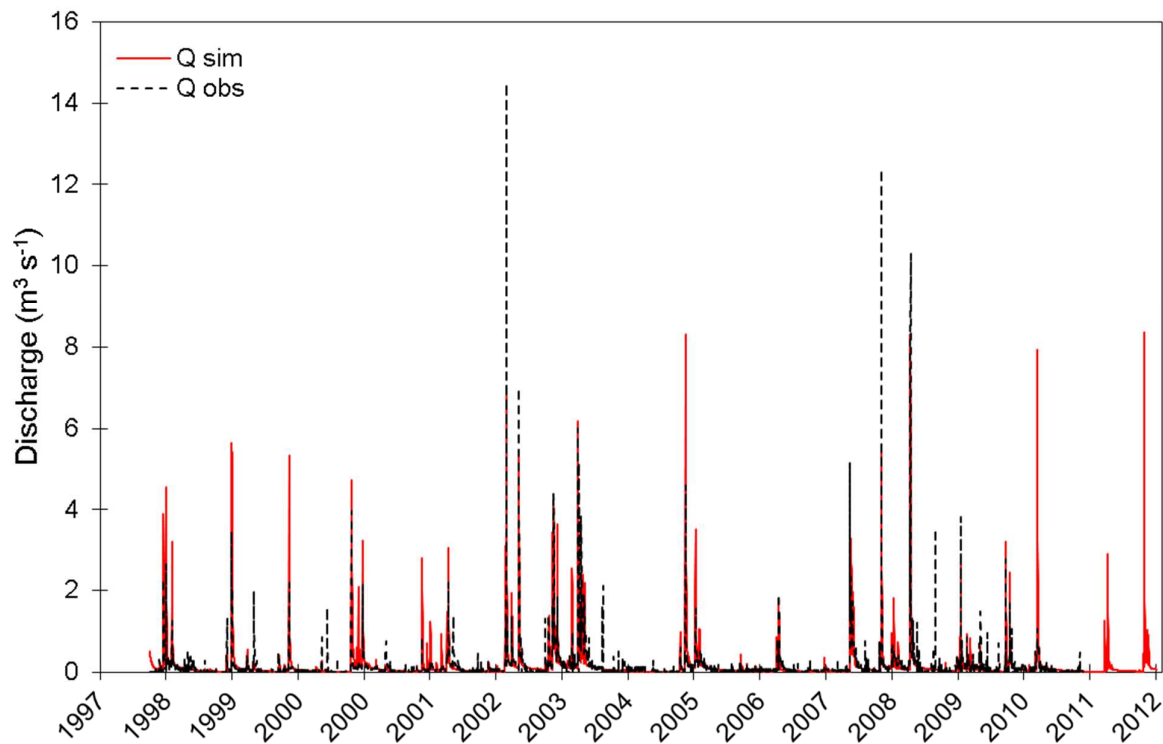
PC	Eigenvalues	%Variation	Cum.%
1	2.51	50.2	50.2
2	1.94	38.7	88.9
3	0.32	6.4	95.3
4	0.187	3.7	99.1
5	0.0474	0.9	100.0

Variable	PC1	PC2	PC3	PC4	PC5
Area	-0.497	0.410	-0.262	-0.228	0.681
Regulation	-0.590	0.192	-0.277	-0.176	-0.712
Armoring	0.058	-0.648	-0.739	-0.143	0.100
Droughts	0.522	0.321	-0.107	-0.775	-0.112
Floods	0.359	0.521	-0.545	0.544	-0.079

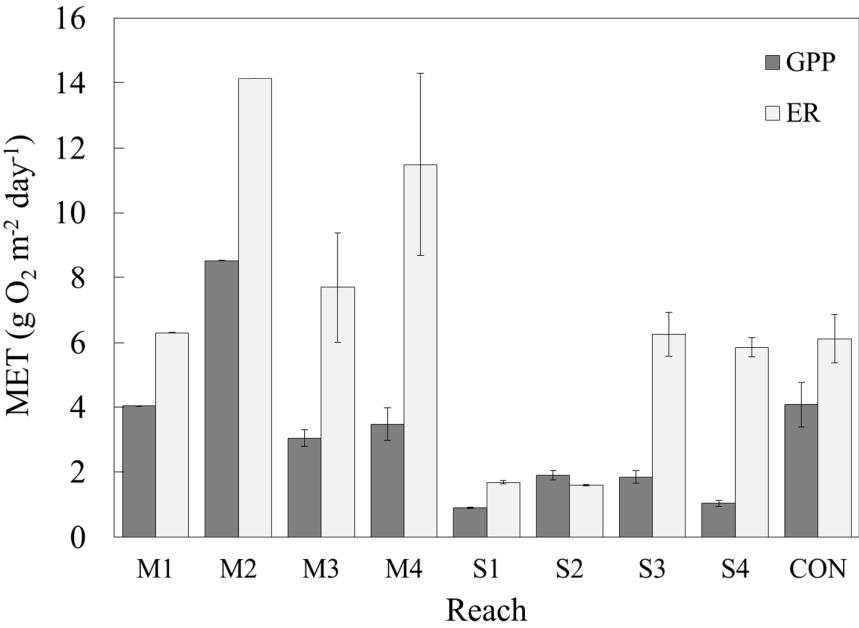
Eigenvectors (Coefficients in the linear combinations of variables making up PC's):



**Figure S1. Principal Components Analysis (PCA) ordering the 9 studied reaches according to their physico-chemical characteristics.** See table S1 for data and units, methods outline, and variable codes. Data was  $\log_{10}(X+1)$  transformed and normalized, and the variable  $\text{NO}_3^-$  was excluded from this analysis due to high correlation ( $\rho > 0.95$ ) with TDN. PC1 axis explains 39.3 % of the variation, PC2 explains 28.3%. M = Montsant river; S = Siurana river; CON = confluence of Montsant and Siurana rivers.



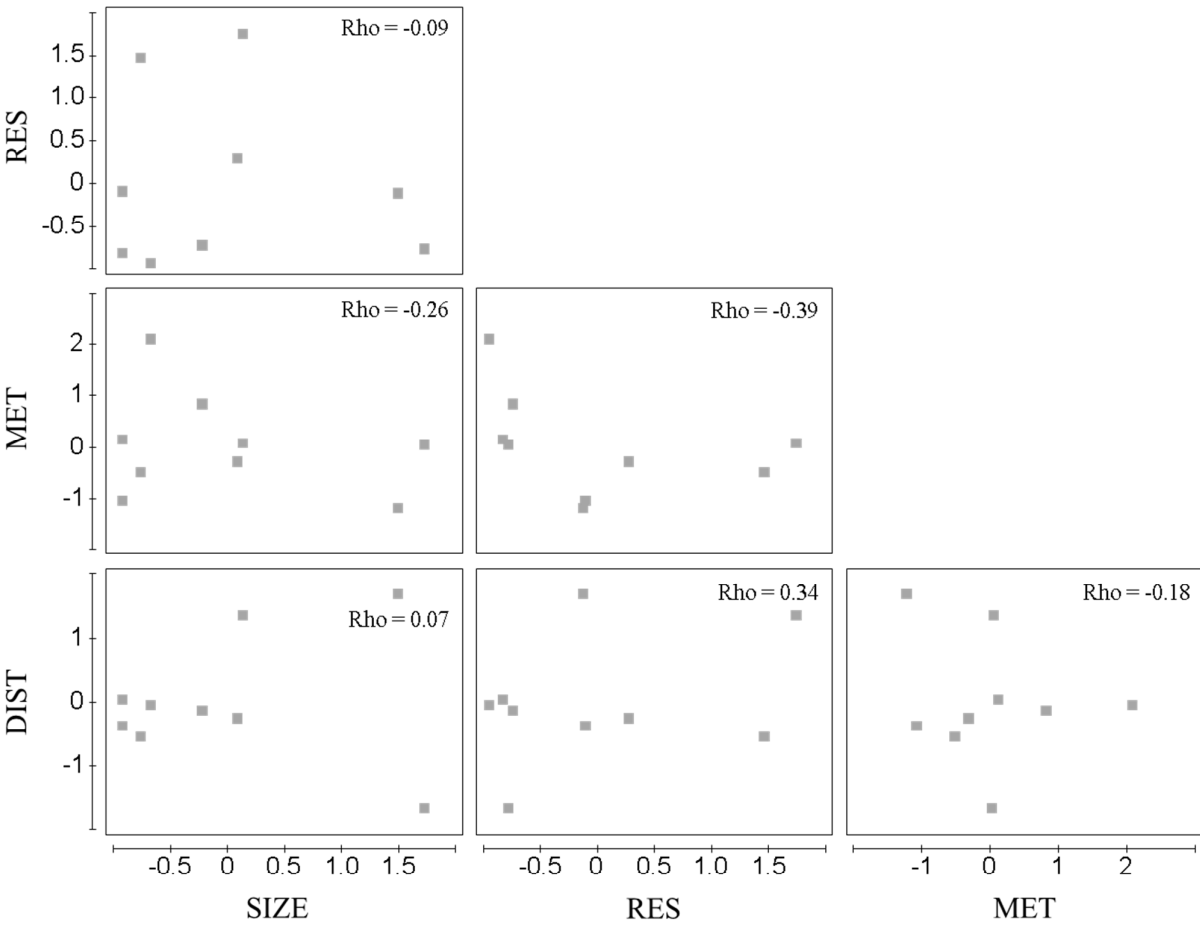
**Figure S2. Observed ( $Q_{obs}$ ) versus simulated flow ( $Q_{sim}$ ) at the Siurana reservoir.** The TETIS model includes an automatic calibration tool, based on the SCE-UA algorithm (Duan, Sorooshian & Gupta 1992). The model is calibrated by adjusting up to 9 coefficients (i.e., correction factors). Each correction factor multiplies a parameter map, allowing calibrating the model without losing the spatial structure of the parameter, and reducing drastically the number of parameters to be calibrated. We included an additive stochastic error term (Vrugt *et al.* 2002) to account for measurement errors. TETIS is a dynamic conceptual model well-suited to describe the hydrological cycle in Mediterranean watersheds (Medici *et al.* 2008). For more information on this model, see Francés *et al.* (2007), Vélez *et al.* (2009), and Bussi *et al.* (2013).



**Figure S3. Ecosystem metabolism (MET) across the studied reaches.** MET was calculated as the sum of gross primary production (GPP, in dark gray) and ecosystem respiration (ER, in white). Error bars represent standard deviations. Ecosystem metabolism was estimated in the 9 study reaches by means of the two-station technique (Odum 1956; Marzolf, Mulholland & Steinman 1994; Reichert, Uehlinger & Acuña 2009). At all stations, dissolved oxygen and temperature were recorded in 10-min intervals during 4 days with optical dissolved oxygen probes YSI 6150 connected to YSI 600 OMS V2 multiparameter sonde (YSI Inc., Yellow Springs, Ohio, USA). The probes were deployed in the thalweg of the stream, about 5 cm below the water surface. Before deployment, the dissolved oxygen sensors were calibrated according to the manufacturer’s manual. After the field measurements, sonde-to-sonde variability was determined by simultaneously immersing the probes in a thermo-regulated and aerated water bath ( $\pm 0.1$  °C). The temperature of the water bath was successively adjusted to 20, 18, 16, 14, 12, 10, 8 and 6 °C and dissolved oxygen recorded every 30 s. Saturation concentration of dissolved oxygen was calculated using recorded temperatures and barometric pressure from a meteorological station of the Catalan Meteorological Service (XEMA). Deviations from the calculated saturation concentrations were determined and used to correct the

field dissolved oxygen records. Estimates of reaeration coefficients were based on the decline of dissolved O<sub>2</sub> concentration after dusk (Hornberger & Kelly 1975). Daily reaeration coefficients were the means of values from the nights before and after the day of interest. Net metabolism rate (NM, expressed as g O<sub>2</sub> m<sup>-2</sup> min<sup>-1</sup>) was calculated from the dissolved O<sub>2</sub> concentration, water temperature, atmospheric pressure, gas-exchange rate, and flow velocity using the open-channel two-station techniques originally developed by (Odum 1956) and further modified by (Reichert *et al.* 2009). Three daily metabolic parameters were based on NM: net ecosystem metabolism (NEM), ecosystem respiration (ER), and gross primary production (GPP). NEM was calculated as the sum of NM over 24 h, ER as the sum of NM during the dark period and respiration rates during the light period (calculated as the linear interpolation between the NMs of sunrise and sunset of the nights before and after the day of interest), and GPP as the difference between NEM and ER. M = Montsant river; S = Siurana river; CON = confluence of Montsant and Siurana rivers.





**Figure S4.** *Scatter plots showing the correlations among the hypothesized environmental controls of FCL (DIST, MET, RES, SIZE). Spearman's ranks correlations (Rho) are shown.*

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