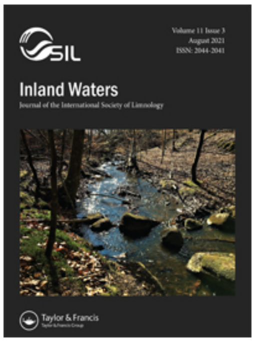

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Impacts of damming and climate change on the ecosystem structure of headwater streams: a case study from the Pyrenees

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ABSTRACT
Climate change, damming, and metal pollution are among the main anthropogenic threats to headwater streams. We designed a case study to assess how these stressors impact the ecosystem structure of headwater streams by using the biofilm and macroinvertebrate communities of a Pyrenean stream. We observed a strong seasonal pattern in the stream that interacted with the analysed stressors by having synergistic, but also antagonistic, responses on the ecosystem structural parameters. Both damming and a decrease in precipitation reduced the water flow of the stream and increased its temperature, which promoted an increase in algal and macroinvertebrate biomass at the expense of the biodiversity of their communities, a situation expected to worsen in a climate change context. The decrease in precipitation also increased the concentration of metals and metalloids in the water column and in biofilms, but the water diversion from damming reduced their contributions downstream. The maintenance of an adequate ecological flow in dam-impounded streams is encouraged to overcome these impacts in the current climate change context. More field studies are needed to assess how multiple anthropogenic stressors interact and threaten the ecosystem integrity in a realistic and applied context.

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biofilm community; climate change; conservation ecology; damming; ecotoxicology; macroinvertebrates

Introduction

Freshwater biodiversity is highly threatened by many human-mediated processes and stressors such as pollution, habitat destruction, flow modification, overextraction of water, overfishing, alien species introduction, and climate change (Revenga et al. 2005, Dudgeon et al. 2006, Dudgeon 2010, Ormerod et al. 2010). Therefore, rivers and streams are currently among the most exploited ecosystems in the planet (Loh et al. 2005). These threats can cause changes in the composition of the biotic community of a stream, usually by simplifying and impoverishing its biodiversity and other structural components (Maddock 1999), reducing the ability of the stream to recover from disturbances and stressors and affecting ecosystem functioning (Bona et al. 2008, Sabater and Tockner 2009). If the aim is to conserve, manage, and, if needed, restore streams while at the same time assuring goods, services, and energy to human populations, threats that compromise the structure and function of these ecosystems must be assessed (Freeman et al. 2007).

One of the main threats to the conservation of stream ecosystems is the construction of dams and weirs as renewable energy sources promoted to reduce greenhouse gas emissions and to supply electricity to >940 million people, especially in low-income regions (World Bank 2018, Zarfl et al. 2019). Despite their social benefits, dams have many potential serious and long-term downsides for ecosystems: habitat degradation and fragmentation, biodiversity loss, ecosystem services erosion, changes in hydrology and sediment transport, flow reduction, water temperature increase, and deterioration of water quality, among others (Bednarek 2001, Lessard and Hayes 2003, Zarfl et al. 2019). Water flow is considered a master variable on stream ecosystems because it structures the habitat, influences physical and chemical parameters, and controls population and community dynamics. Because stream communities are adapted to a natural water flow variability, alterations from damming can impact the structure and function of these ecosystems (Bednarek 2001, Mor et al. 2018).

Since the 20th century, the hydromorphological alteration of streams has been coupled with the proliferation of industries and mining, increasing the metal and metalloid concentrations of their waters and sediments (Colas et al. 2013). These pollutants can bioaccumulate

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in the organisms, transforming into persistent metallic compounds with high toxicity (Jin 1992), but they can also accumulate in the sediments retained by a dam, impairing the local biological communities and threatening distant downstream communities with flush-outs or dam removals (Colas et al. 2013). Current research on metal pollution is integrating ecological principles on dam design and implementation to properly assess the impacts of metals and metalloids on ecosystems and biological communities (Gessner and Tlili 2016). Therefore, evaluating metal pollution in streams affected by damming can provide a better understanding of how metals and metalloids can impact biological communities in hydrologically altered streams.

Current pressures on stream ecosystems will be exacerbated by the effects of climate change, severely impacting their ecosystem structure and function (Walther et al. 2002, Parmesan and Yohe 2003, Sabater and Tockner 2009, Muñoz-Mas et al. 2018). Headwater streams, a type of lotic ecosystem especially vulnerable to climate change, are first- and second-order streams characterized by low water temperatures, distinct seasonal and daily flow variations, oligosalinity, and singular hydrological and morphological conditions (Bona et al. 2008). The ecosystem structure of headwater streams is driven by a marked seasonal flow variability (Scarsbrook and Townsend 1993, Biggs et al. 2005); the hydrological regime on these systems is regulated by processes of snow accumulation and snowmelt that drive low and uniform winter flows and highwater flows during the snowmelt in spring and early summer (López-Moreno and Beniston 2009, Sanmiguel-Vallelado et al. 2017). Climate change is expected to alter this seasonal pattern as the precipitation regime and snow cover decrease (Cramer et al. 2018). Because freshwater organisms in headwater streams are adapted to a cold and natural flow (Poff et al. 1997), these alterations caused by climate change can strongly impact the ecosystem structure of headwater streams (Perkins et al. 2010). Climatic models of the Pyrenees, a mountain range located in the northwest Iberian Peninsula (western part of the Mediterranean Basin), predict a decrease in precipitation between 10.7% and 14.8% and a mean increase in temperatures of between 2.8 and 4 °C by the end of the 21st century (López-Moreno et al. 2008), as well as a decrease in the maximum level of accumulated snow (López-Moreno et al. 2009). Therefore, this mountain range is a suitable area to research how climate change can impact the ecosystem structure of headwater streams.

Collectively, these anthropogenic threats can stress the biotic communities of headwater stream ecosystems. Ecosystem stress is defined as a condition caused by environmental factors that bring organisms near the edges of the reference ranges for a determined ecological function, causing communities to adapt their ecological niches to new environmental conditions (Straalen 2003, Steinberg 2012). Multiple natural and anthropogenic stressors can occur simultaneously, causing complex responses by the biotic communities that can be additive if the effects of multiple stressors equal the sum of their individual effects, or multiplicative if the resultant effect is greater (synergism) or lesser (antagonism) than the sum of their individual effects (Piggott et al. 2015, Jackson et al. 2016). In this regard, climate change could interact with other stressors related to flow reduction from damming or increasing metal concentrations to affect the ecosystem structure of headwater streams.

Understanding how these multiple anthropogenic stressors interact to impair the ecosystem structure of headwater streams is key for the effective management and conservation of these ecosystems. In this context, this research is a case study of a Pyrenean headwater stream affected by the presence of a hydroelectric dam and by metal pollution from an old antimony (Sb) mine in a climate change scenario. The main focus of this case study is to assess the seasonal response of the ecosystem structure to the presence of a dam, reduced precipitation, and metal pollution. We hypothesized that damming and reduced precipitation would decrease the water flow of the stream and increase its temperature, affecting other abiotic parameters and the concentration of metal and metalloid pollutants, with negative consequences for the ecosystem structure of the stream. Further, we expect these stressors to be modulated by seasonal variability, with potential for multiplicative synergistic effects on the stream ecosystem structure.

Materials and methods

Study area and data collection

The study area is a small Pyrenean headwater stream named Catllar, an influent of the Ter River, in Villalonga del Ter, Catalonia (northeast Iberian Peninsula), located between 1200 and 1600 m a.s.l. We selected 4 sampling points (Upst 1, Upst 2, Down 1, and Down 2) spaced approximately equidistantly and representative of the altitudinal zonation of the stream. A hydroelectric dam located between the Upst 2 and Down 1 sampling points divides the stream. It deviates most of the upstream water to a hydroelectric station, and downstream water comes mainly from the adjacent west stream. An abandoned Sb mine was present west of the Upst 1 sampling point (Fig. 1), a source of metals

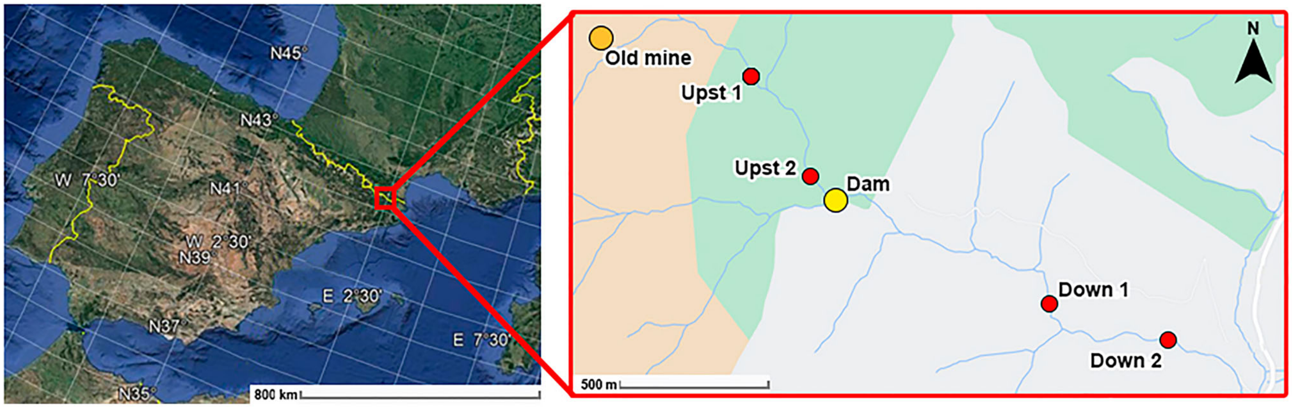

Figure 1. Catllar stream basin with its main tributaries and 4 sampling points (Upst 1, Upst 2, Down 1, and Down 2: red dots), the dam (yellow dot), and the abandoned mine (orange dot). Catllar stream is a tributary of the Ter River located in Catalonia, northeast Iberian Peninsula (red square).

and metalloids in the stream. We collected water samples and physical and chemical parameter measures and sampled biofilms and macroinvertebrates at each sampling point each season during 2 years from spring 2018 to winter 2020, with the exception of summer 2018, resulting in 7 samplings per point.

Biofilm sampling and analyses

Biofilm samples were taken by scraping 25 cm^2 of the surface of random stones in the riverbed and storing in 30 mL vials with river water. Four repetitions for each sampling were undertaken for algal community characterisation, chlorophyll *a* (Chl-*a*) concentration (algal biomass), total biomass expressed as ash free dry mass (AFDM), and metal and metalloid bioaccumulation. Chl-*a* and AFDM samples were frozen until analysis. For algal community analysis, the fresh sample was preserved with a 15 µL drop of 4% formalin and chilled until analysis. The metal bioaccumulation samples were frozen, lyophilised, and then microwave-digested using vials with nitric acid and hydrogen peroxide inside Teflon pressure vessels with water and hydrogen peroxide. The metal concentrations in the digested biofilm samples were then measured in an inductively coupled plasma mass spectrophotometer (ICP-MS).

To analyse the Chl-*a* concentration and AFDM of the biofilm samples, we void-filtered using 47 mm diameter glass-fibre filter papers. The solid residue was put in a glass container with 20 mL of 90% acetone to extract Chl-*a* and then preserved cold and in the dark for 24 h. The sample was then sonicated (J.P. Selecta, Barcelona, Spain) for 2 min to promote cell lysis and improve Chl-*a* extraction and then again void-filtered using another pre-dried and weighed filter paper. Extra acetone was used to wash all the biofilm, and the total volume used was considered for the calculations. The filtered acetone contained the Chl-*a* of the sample, which was measured using a spectrophotometer (Shimadzu UV-1800) following the methodology described by Jeffrey and Humphrey (1975). The residue on the paper was dried at 50 °C for a week in a drying oven (Raypa DOD-20, Barcalona, Spain) until a constant weight was achieved, and then weighed with an analytical scale (Sartorius Practum 124-1S, Göttingen, Germany) to measure the dry mass of the biofilm. It was then dehydrated at 450 °C for 4 h and weighed again to measure AFDM. These values were used to calculate the percentage of organic matter (proportion of dry mass weight from the total weight) and the autotrophic index (AFDM divided by the Chl-*a* concentration) of the samples.

To identify the algal community composition of the biofilms, a 15 µL drop of the scraped samples was mounted under a 22 mm × 22 mm slide cover and observed with an optic microscope (Nikon E600, Tokyo, Japan) at 400× to identify algae (mainly diatoms) to a genus taxonomic level, counting up to 400 cells. We measured 25 cells per genus to estimate its biovolume from the shape equations proposed by Hillebrand et al. (1999). Total algal biovolume per sample was calculated by multiplying the cell abundance by the calculated biovolume for each genus and then expressed as areal density ($\mu m^3/cm^2$). Shannon diversity index using the genera count was also calculated.

Macroinvertebrate samplings and analyses

Macroinvertebrate samplings were based on the quantitative macroinvertebrate sampling protocols from the Catalan Water Agency (ACA 2006) and the Spanish Ministry of Agriculture, Food and Environment

(MAGRAMA 2013). For each sampling point, a coverage percent estimation for each type of microhabitat substrate was assessed. Using a 25 cm wide and 500 µm mesh size hand net, we collected 10 sampling units covering 0.125 m^2 at each point proportionally distributed for each microhabitat type: 1 per each 10% of microhabitat coverture plus an extra half sampling unit for minority habitats (<5%). Samples were stored in plastic containers with 80% ethanol until processing and analysis at the laboratory.

Macroinvertebrates were identified using a stereoscopic microscope (Optika SZR-10) by means of identification keys (Campaioli et al. 1999, Malicky 2004, Tachet et al. 2010, Oscoz et al. 2011). Taxonomic resolution was based on the Iberian Biological Monitoring Working Party (IBMWP) biological quality index (Alba-Tercedor et al. 2002) in which taxa were identified up to family level, except for Nematoda, Nematomorpha, Oligochaeta, Ostracoda, Hydracarina, and Collembola. For each sample, a fraction of at least 300 individuals was identified and counted. All individuals of the taxa not present in the analysed fraction but found in the rest of the sample were also counted. Abundance of each taxon was calculated by extrapolating the abundance of all individuals present in the analysed fraction plus the number of individuals in the remaining sample. Considering the sampled area, results were expressed as density (individuals/m^2). The dry mass of each taxon in the samples was assessed using a representative subsample of each taxon dried at 60 °C until constant weight using a drying oven (Raypa DOD-20) and weighed using an analytical scale (Sartorius Practum 124-1S). For biomass calculations, subsamples that did not reach the detection limit (DL = 1 mg) had a value assigned of 0.7 DL. Samples that had 30% of the taxa under the detection limit were not considered (Bennett et al. 2000). Total weight of each subsample was divided by the total number of individuals, and these values were multiplied by the abundance of each taxon and expressed as mg of dry mass/m^2. Finally, 3 biological quality indexes for macroinvertebrates were calculated: IBMWP (Alba-Tercedor et al. 2002), Iberian Average Score per Taxon (IASPT; Armitage et al. 1983), and the Ephemeroptera, Plecoptera, and Trichoptera (EPT) index (Lenat 1988).

Physical and chemical parameters

We measured water temperature, conductivity, pH, and oxygen concentration at each sampling point by using a multiprobe (Geotech, USA), and the water flow by using the mass balance method (Hudson and Fraser 2005). We also analysed the metal (chromium [Cr], nickel [Ni], copper [Cu], zinc [Zn], and cadmium [Cd]) and metalloid (arsenic [As] and Sb) concentrations in the water by filtering 5 mL of stream water through nylon fibre filters, adding 15 µL of nitric acid to the sample to avoid metal precipitation, and then detecting using an ICP-MS. Hydromorphological characteristics of the stream were assessed at each sampling point by applying the Fluvial Habitat Index (FHI; Pardo et al. 2002, ACA 2006). Air temperature, precipitation, wind speed, and atmospheric pressure data were obtained in situ from a meteorological station that measured these parameters daily from spring 2018 to winter 2020. Additionally, data from the water flow of the Ter River were obtained in the nearest water flow station at Sant Joan de les Abadesses and used to obtain the monthly average flow between 2009 and 2020 to understand the seasonality of the water flow in this Pyrenean area.

Statistical analyses

Statistical analyses were performed using RStudio 1.2.5033 software (RStudio Team 2019). Because the summer 2018 sampling, and consequently a replicate for this season, were lacking, we removed the summer data from the statistical analyses and considered summer 2019 information as descriptive only. Biofilm, macroinvertebrate, physical, and chemical variables were checked, and if data did not meet normality and homoscedasticity assumptions they were transformed using logarithms, square roots, or Box-Cox transformations (Box and Cox 1964) as best suited. Precipitation regimes were different between seasons and years, so to assess the potential effects of climate change, we used the average daily and accumulated precipitation data results from an in situ meteorological station, plus the water flow data from the Sant Joan de les Abadesses flow station, to categorize each season as wet (spring 2018, autumn 2018, and winter 2020) or dry (spring 2019, autumn 2019, and winter 2019), when compared one to another. To account for the influence of seasonality and assess the impact of damming and the different precipitation regimes on the abiotic and biotic structural parameters of the Catllar stream, variables were grouped by season (spring, autumn, and winter), relative position from the dam (Upst 1 and Upst 2 = upstream; Down 1 and Down 2 = downstream), and precipitation regime (dry or wet). We then applied a linear mixed-effects model (LME) for each variable by considering the season, the relative position from the dam, and the precipitation regime as fixed effects, and the sampling point (Upst 1, Upst 2, Down 1, and Down 2) as the random effect.

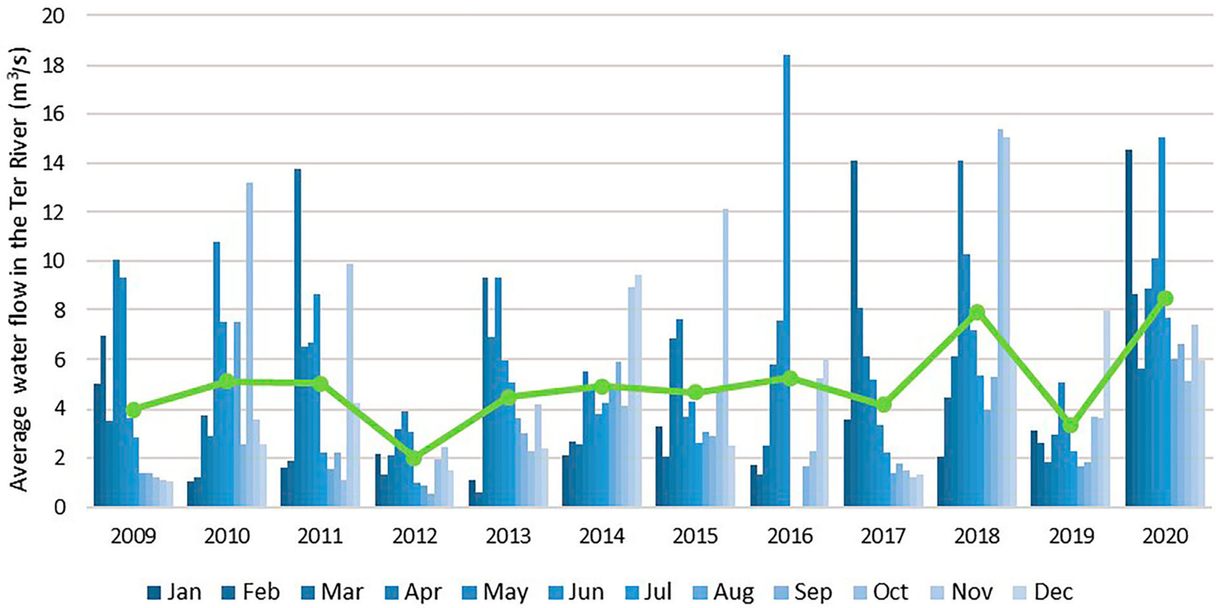

Figure 2. Average values of water flow of the Ter River at the Sant Joan de les Abasesses flow station during 2009–2021 for each month (blue columns) and each year (green line).

To assess the differences in the biofilm and macroinvertebrate community composition between seasons, relative position from the dam, and precipitation regime, we performed an analysis of similarities (ANOSIM) using the *vegan* R package (Oksanen et al. 2019). Algal genera biovolumes and macroinvertebrate family biomasses, excluding those representing <1% of the total biomass, were used as dependent variables. In addition, we performed a principal component analysis (PCA) of the biofilm and macroinvertebrate data for each sampling with the objective of observing differences in their community composition within and between years.

Results

Seasonality

Low average daily temperatures were observed all year, with a maximum of 17 °C in summer 2019 and a minimum of 5.3 °C in winter 2019. Precipitation regimes were different between seasons and years, with a maximum of 546.2 mm of accumulated rain in autumn 2018 and a minimum of 44.6 mm in winter 2019. This Pyrenean precipitation variability was reflected in the San Joan de les Abadesses flow station with a high-water flow variability caused by high or low precipitation regimes. From 2016 to 2020, the water flow was highly variable seasonally and interannually (Fig. 2), allowing us to compare each season as wet or dry during the 2 years of this study (2018–2020). In the Catllar stream, this strong seasonal pattern was reflected in the water flow and temperature variability. Water temperature, conductivity, oxygen concentration, pH, and flow differed among seasons (Table 1 and 2). Metal and metalloid concentrations of Cr, Zn, As, Cd, and Sb in the stream water, as well as the concentration of Cr, Zn, and Cd in biofilms, also differed among seasons (Table 3 and 4).

The ANOSIM analysis showed that the community composition of algal genera in the biofilms ($n = 16$) and of macroinvertebrate families ($n = 23$) was significantly different among seasons (algal genera: $R = 0.217$, $p = 0.004$; macroinvertebrate families: $R = 0.519$, $p = 0.001$) but not relative position to the dam or precipitation regimes. The PCA analysis showed that, for the algal community, the highest diversity was found during winter (Fig. 3). In the dry winter of 2019, we observed a dominance of the diatom genera *Fragilaria*, *Diatoma*, *Cyclotella*, and *Cymbella*, which were dominant also during the dry spring and summer of 2019. In the wet winter of 2020, we predominantly found *Hydrurus*, *Amphora*, *Cocconeis*, and *Oscillatoria*. It was remarkable that, during winter, a bloom of the macroalgae *Hydrurus foetidus* (Villars) Trevisan 1848 was observed in the riverbed. For the macroinvertebrate community composition (Fig. 4), most of the diversity was found in winter and spring, with most families shared between both seasons.

Considering the ecosystem structure parameters, the AFDM and Chl-*a* concentration in biofilms was highest during winter, coinciding with the macroinvertebrate abundance peak (Table 1 and 2). We observed that

Table 1. Average (standard deviation) of water temperature, conductivity, oxygen concentration, pH, flow, and Fluvial Habitat Index (FHI); biofilm algal biovolume, biomass, ash free dry mass (AFDM), algal Shannon diversity, algal genera richness, autotrophic index, and percentage of organic matter; and macroinvertebrate biomass, abundance, families, and the Iberian Biological Monitoring Working Party (IBMWP), Iberian Average Score per Taxon (IASPT), and Ephemeroptera, Plecoptera, and Trichoptera (EPT) quality indices grouped by season, relative position to the dam and precipitation regime. The number of samples indicates how many seasonal replicates were used for the calculations. Each seasonal replicate includes the averaged values of the 4 sampling points along the stream.

	Season				Position		Precipitation regime	
Parameter	Spring	Summer	Autumn	Winter	Upstream	Downstream	Wet	Dry
Water								
Temperature (°C)	7.6 (1.4)	15.1 (1.1)	4.4 (1.1)	5.7 (0.8)	5.2 (1.6)	6.6 (1.6)	5.9 (1.5)	6.0 (1.9)
Conductivity (µS/cm)	153 (3.9)	192 (26.1)	161 (29)	157 (37.8)	128 (14.2)	186 (18.2)	145.8 (28.6)	168.8 (33.4)
Oxygen (mg/L)	9.5 (0.3)	8.1 (0.15)	10.5 (0.2)	10.1 (0.1)	10.1 (0.4)	9.9 (0.4)	10.0 (0.4)	10.0 (0.5)
pH	8.1 (0.2)	8.0 (0.04)	7.9 (0.2)	7.8 (0.2)	7.9 (0.2)	8.0 (0.2)	8.0 (0.2)	7.9 (0.2)
Flow (m^3/s)	0.2 (0.1)	0.07 (0.01)	0.1 (0.1)	0.2 (0.1)	0.2 (0.1)	0.1 (0.1)	0.2 (0.1)	0.1 (0.0)
FHI	69 (4.2)	74 (1.0)	66 (2.1)	73 (4.0)	68.2 (4.1)	70.7 (4.5)	69.6 (3.7)	69.3 (5.2)
Biofilms								
Algal biovolume ($\mu m^3/cm^2$)	1.5×10^8 (8.1×10^7)	8.4×10^7 (1.1×10^8)	3.8×10^8 (2.5×10^8)	7.1×10^8 (5.4×10^8)	3.4×10^8 (2.4×10^8)	4.8×10^8 (5.3×10^8)	4.8×10^8 (5.4×10^8)	3.4×10^8 (2.1×10^8)
Biomass (µg Chl-*a*/cm^2)	18.1 (4.7)	28.4 (13.2)	29.0 (14.8)	182 (109)	73.2 (99.7)	80.0 (97.5)	57.6 (42.3)	95.6 (130)
AFDM (mg/m^2)	2297 (1756)	464) (175	653 (215)	6204 (4596)	2912 (3526)	3190 (3812)	2454 (2701)	3648 (4358)
Algal Shannon diversity	1.9 (0.4)	1.3 (0.5)	2.1 (0.2)	2.1 (0.4)	2.1 (0.2)	1.9 (0.4)	2.1 (0.3)	2.0 (0.4)
Algal genera richness	7.8 (2.0)	4.7 (1.9)	11.0 (1.2)	10.0 (1.7)	10.0 (1.3)	9.7 (2.7)	10.2 (2.0)	9.0 (2.1)
Autotrophic index	127 (81.2)	16.8 (2.9)	161 (29.8)	157 (37.8)	128 (14.2)	186 (18.2)	145 (28.6)	168 (33.4)
% Organic matter	34.1 (7.7)	43.3 (9.6)	39.4 (19.4)	54.4 (33.3)	33.7 (21.4)	51.5 (23.7)	38.4 (20.3)	46.9 (27.0)
Macroinvertebrates								
Biomass (mg/m^2)	2931 (768)	2270 (665)	1257 (201)	2839 (811)	2162 (944)	2523 (1040)	2331 (870)	2354 (1133)
Abundance (ind/m^2)	3273 (335)	3342 (995)	3317 (598)	4206 (956)	3716 (787)	3481 (809)	3272 (543)	3925 (891)
Families	37.2 (1.7)	37 (2.7)	32.1 (1.6)	33.1 (3.1)	34.0 (3.8)	34.3 (2.3)	35.3 (3.3)	33.1 (2.7)
IBMWP	234 (6.8)	223 (12.8)	206 (9.2)	212 (14.6)	216 (18.1)	218.8 (13.2)	221 (16.6)	214 (14.2)
IASPT	6.3 (0.2)	6.1 (0.1)	6.4 (0.2)	6.4 (0.2)	6.4 (0.3)	6.4 (0.1)	6.3 (0.2)	6.5 (0.2)
EPT	17.9 (0.6)	16.3 (0.8)	16.6 (0.9)	16.6 (1.3)	16.7 (1.3)	17.4 (0.8)	17.2 (1.0)	16.9 (1.3)
No. samples	2	1	2	2	6	6	6	6

Table 2. Results of the linear mixed-effects model (LME) for the water temperature, conductivity, oxygen concentration, pH, flow, and Fluvial Habitat Index (FHI); biofilm algal biovolume, biomass, ash free dry mass (AFDM), algal Shannon diversity, algal genera richness, autotrophic index, and percentage of organic matter; and macroinvertebrate biomass, abundance, and families, and the Iberian Biological Monitoring Working Party (IBMWP), Iberian Average Score per Taxon (IASPT), and Ephemeroptera, Plecoptera, and Trichoptera (EPT) quality indices: effects of season, relative position to the dam and precipitation regime (P.R.). The degrees of freedom are 1 for position, precipitation regime, and their interaction, and 2 for season and its interactions with position and precipitation regime. Variables were transformed using $\log_{10}$, square root, or Box-Cox to reach normality. Significant results ($p < 0.05$) are in boldface.

	Season		Position		P.R.		Season*Position		Season*P.R.		Position*P.R.		Season*Position* P.R.	
	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Water														
Temperature	185.93	**<0.05**	3.52	0.06	0.19	0.66	10.03	**<0.05**	16.11	**<0.05**	4.60	**<0.05**	4.29	0.12
Conductivity	204.46	**<0.05**	386.79	**<0.05**	2539	**<0.05**	4.11	0.13	2072	**<0.05**	175.37	**<0.05**	3.50	0.17
Oxygen	272.10	**<0.05**	2.36	0.12	0.12	0.73	4.08	0.13	7.24	**<0.05**	6.72	**<0.05**	2.22	0.33
pH	31.32	**<0.05**	4.93	**<0.05**	3.40	0.06	0.28	0.87	31.71	**<0.05**	0.03	0.86	2.11	0.35
Flow	10.38	**<0.05**	7.10	**<0.05**	112.46	**<0.05**	5.17	0.07	6.43	**<0.05**	0.46	0.50	5.37	0.07
FHI	19.58	**<0.05**	3.98	**<0.05**	0.07	0.79	1.38	0.50	8.74	**<0.05**	4.53	**<0.05**	1.73	0.42
Biofilms														
Algal biovolume	22.45	**<0.05**	0.94	0.33	0.41	0.52	4.46	0.12	7.55	**<0.05**	2.94	0.09	3.29	0.19
Biomass	231.24	**<0.05**	0.02	0.89	19.11	**<0.05**	1.50	0.47	30.81	**<0.05**	0.52	0.47	7.73	**<0.05**
AFDM	28.15	**<0.05**	0.08	0.78	0.44	0.51	7.38	**<0.05**	13.21	**<0.05**	3.35	0.06	1.80	0.41
Algal Shannon diversity	0.92	0.63	1.40	0.23	0.18	0.67	5.80	**0.05**	0.91	0.63	1.32	0.25	1.13	0.57
Algal genera richness	22.45	**<0.05**	0.94	0.33	0.41	0.52	4.46	0.11	7.55	**<0.05**	2.94	0.09	3.29	0.19
Autotrophic index	37.09	**<0.05**	0.01	0.93	2.52	0.11	10.21	**<0.05**	6.75	**<0.05**	6.81	**<0.05**	4.62	0.10
% Organic matter	0.79	0.67	4.95	**<0.05**	1.06	0.30	2.70	0.26	1.89	0.39	2.57	0.11	2.39	0.30
Macroinvertebrates														
Biomass	108.20	**<0.05**	4.71	**<0.05**	0.37	0.54	1.96	0.37	21.70	**<0.05**	5.25	**<0.05**	1.84	0.40
Abundance	13.44	**<0.05**	0.34	0.56	7.76	**<0.05**	0.34	0.84	6.08	**<0.05**	0.10	0.76	2.75	0.25
Families	45.71	**<0.05**	0.26	0.61	10.90	**<0.05**	6.42	**<0.05**	16.48	**<0.05**	0.06	0.78	1.58	0.45
IBMWP	42.27	**<0.05**	0.28	0.59	4.21	**<0.05**	1.86	0.39	14.80	**<0.05**	0.51	0.47	1.42	0.49
IASPT	4.14	0.13	0.16	0.69	7.22	**<0.05**	7.91	**<0.05**	2.75	0.25	2.75	0.10	0.46	0.79
EPT	13.46	**<0.05**	4.16	**<0.05**	0.44	0.09	4.79	0.09	10.83	**<0.05**	1.06	0.30	0.86	0.65

Table 3. Average (standard deviation) of metal (chromium [Cr], nickel [Ni], copper [Cu], zinc [Zn], and cadmium [Cd]) and metalloid (arsenic [As] and antimony [Sb]) concentrations in water and in dry biofilm grouped by seasons, relative position to the dam and precipitation regime. The number of samples indicate how many seasonal replicates were used for the calculations. Each seasonal replicate includes the averaged values of the 4 sampling points along the stream.

	Season				Position		Precipitation regime	
Metals	Spring	Summer	Autumn	Winter	Upstream	Downstream	Wet	Dry
Water (µg/L)								
Cr	0.2 (0.1)	2.8 (0.4)	1.3 (0.3)	1.0 (0.8)	0.9 (0.9)	0.8 (0.9)	0.8 (0.8)	0.9 (1.0)
Ni	0.5 (0.3)	2.7 (1.8)	0.8 (0.7)	0.6 (0.5)	0.5 (0.3)	0.8 (0.7)	0.7 (0.4)	0.5 (0.6)
Cu	0.8 (0.9)	3.9 (1.9)	1.5 (0.9)	1.3 (1.0)	1.3 (1.1)	1.1 (0.9)	1.3 (0.9)	1.1 (1.1)
Zn	1.7 (1.1)	15.2 (7.9)	3.1 (3.4)	11.6 (14.0)	3.4 (5.1)	7.6 (11.9)	3.9 (3.9)	7.0 (12.5)
As	8.2 (5.9)	28.6 (26.9)	8.3 (7.2)	11.1 (7.2)	14.1 (6.1)	4.3 (3.1)	7.1 (5.7)	11.3 (7.3)
Cd	0.00 (0.01)	0.05 (0.02)	0.02 (0.01)	0.02 (0.01)	0.01 (0.01)	0.02 (0.02)	0.01 (0.01)	0.02 (0.01)
Sb	0.1 (0.1)	1.6 (1.0)	0.3 (0.1)	0.2 (0.1)	0.2 (0.1)	0.2 (0.2)	0.2 (0.2)	0.2 (0.1)
Dry biofilm (µg/g)								
Cr	607 (608)	238 (110)	323 (169)	160 (93.5)	418 (435)	308 (379)	541 (516)	186 (96.5)
Ni	468 (301)	619 (88.7)	540 (153)	618 (584)	679 (483)	405 (200)	551 (199)	533 (521)
Cu	252 (99.6)	463 (118)	288 (125)	485 (607)	476 (492)	207 (74.5)	259 (93.2)	425 (511)
Zn	867 (343)	1628 (221)	1435 (410)	5738 (10767)	4162 (9074)	1198 (456)	1321 (334)	4039 (9118)
As	826 (549)	1426 (720)	2150 (2299)	1118 (749)	2052 (1877)	677 (526)	1029 (562)	1700 (2050)
Cd	5.5 (3.3)	14.2 (2.4)	10.6 (5.5)	24.8 (37.7)	18.8 (32.1)	8.5 (5.1)	9.6 (5.1)	17.7 (32.4)
Sb	0.8 (0.4)	3.5 (2.6)	0.9 (0.7)	3.4 (6.4)	2.6 (5.4)	0.9 (0.4)	0.9 (0.5)	2.5 (5.4)
No. samples	2	1	2	2	6	6	6	6

water temperature was negatively correlated with algal genera richness in biofilms (Fig. 5). The highest number of macroinvertebrate families and values of the IBMWP and EPT quality indices were observed during spring (Table 1).

Effects of damming and its interaction with the seasonal responses of the ecosystem

Damming altered the physical and chemical characteristics of the downstream section of the stream by reducing water flow and increasing water temperature, conductivity, pH, and the FHI (Table 1 and 2). Water temperature was higher downstream than upstream during all seasons. Metal and metalloid concentrations were also affected by damming, reflected by significantly lower concentration of Ni, Cu, and As in biofilms as well as the As concentration in water in the downstream section of the stream (Table 3 and 4). The presence of the dam itself also impacted the ecosystem structure of the stream by increasing the downstream relative organic matter content of the biofilms, macroinvertebrate biomass, and EPT index (Table 1 and 2). The seasonal pattern of the ecosystem structure of the stream was altered by the presence of the dam. During cold seasons downstream of the dam, the AFDM and autotrophic index of the biofilm were higher and the algal and macroinvertebrate diversity was lower than upstream (Fig. 6).

Effects of the precipitation regime and its interaction with the seasonal responses of the ecosystem

Different precipitation regimes significantly altered the physical and chemical characteristics of the stream by diminishing the water flow during low precipitation regimes, increasing its conductivity and the As concentrations in water and in biofilms. The ecosystem structure was also affected by the different precipitation regimes, with a significant increase of algal biomass (Chl-*a* concentration) and macroinvertebrate abundance as well as a decrease in macroinvertebrate family richness observed during the dry regime. Macroinvertebrate quality indices also differed significantly depending on the precipitation regime, with the exception of EPT (Table 1 and 2).

The precipitation regime had a significant interaction with the seasonal response of all the analysed abiotic variables as well as the concentration of most of the analysed metals and metalloids (Table 1–4, Fig. 7). All ecosystem structure parameters of biofilms and macroinvertebrates (with the exception of algal Shannon index), the organic matter percentage in biofilms, and the IASPT macroinvertebrate index were affected by this interaction (Table 1 and 2). A remarkable pattern was observed within this interaction during dry precipitation regimes in cold seasons, especially in winter, when water flow reduction was coupled with an increase in algal and macroinvertebrate biomass, but also a loss of algal and macroinvertebrate biodiversity (Fig. 7).

Effects of the interaction between damming and the precipitation regime

The interaction between the precipitation regime and the relative position from the dam affected the water temperature, conductivity, oxygen concentration, and FHI as well as concentration of Cr and Sb. It also affected the autotrophic index of the biofilm and the

Table 4. Results of the linear mixed-effects model (LME) for metal and metalloid concentrations in water and dry biofilms: effects of season, relative position to the dam and precipitation regime (P.R.). The degrees of freedom are 1 for position, precipitation regime and their interaction, and 2 for season and its interactions with position and precipitation regime. Variables were transformed using $\log_{10}$, square root, or Box-Cox to reach normality. Significant results ($p < 0.05$) are in boldface.

Water	Season		Position		P.R.		Season*Position		Season*P.R.		Position*P.R.		Season*Position*P.R.	
	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p	χ^2	p
Chromium	65.54	**<0.05**	0.77	0.38	1.76	0.19	4.16	0.13	51.66	**<0.05**	5.21	**<0.05**	8.93	**<0.05**
Nickel	1.00	0.60	1.25	0.26	2.21	0.14	4.09	0.13	7.77	**<0.05**	0.01	0.94	3.53	0.17
Copper	4.13	0.13	0.34	0.56	1.02	0.31	5.02	0.08	5.79	0.06	2.73	0.10	2.08	0.35
Zinc	8.00	**<0.05**	2.51	0.11	0.07	0.79	4.98	0.08	13.46	**<0.05**	0.34	0.56	0.92	0.63
Arsenic	10.07	**<0.05**	83.49	**<0.05**	14.66	**<0.05**	1.09	0.58	40.89	**<0.05**	0.63	0.43	5.14	0.08
Cadmium	14.63	**<0.05**	0.65	0.42	0.13	0.72	3.23	0.20	13.26	**<0.05**	0.00	0.98	0.71	0.70
Antimony	16.10	**<0.05**	0.02	0.88	0.14	0.71	0.94	0.63	5.72	0.06	3.86	**<0.05**	0.14	0.93
Dry biofilm														
Chromium	10.63	**<0.05**	1.29	0.26	10.42	**<0.05**	1.36	0.51	11.22	**<0.05**	1.19	0.28	4.17	0.12
Nickel	1.37	0.50	6.46	**<0.05**	0.99	0.32	3.61	0.16	18.26	**<0.05**	1.95	0.16	9.29	**<0.05**
Copper	0.81	0.67	6.78	**<0.05**	0.42	0.52	1.93	0.38	2.84	0.24	1.64	0.20	1.66	0.44
Zinc	19.24	**<0.05**	3.68	0.06	0.05	0.82	0.16	0.92	10.51	**<0.05**	0.14	0.71	0.76	0.68
Arsenic	4.73	0.09	16.31	**<0.05**	0.01	0.96	0.51	0.78	7.60	**<0.05**	1.38	0.24	4.27	0.12
Cadmium	11.79	**<0.05**	0.60	0.44	0.42	0.52	1.99	0.37	12.17	**<0.05**	0.23	0.63	1.22	0.54
Antimony	3.67	0.16	0.39	0.53	0.35	0.55	1.90	0.39	8.93	**<0.05**	1.26	0.26	0.88	0.64

macroinvertebrate biomass (Table 2 and 4). During the dry precipitation regime, we observed downstream of the dam a higher water temperature and macroinvertebrate biomass, with a lower autotrophic index of the biofilms (Fig. 8).

Discussion

The structure and function of stream ecosystems is mainly driven by the hydrological regime (Maddock 1999). In this case study, we observed that water flow was the main abiotic factor controlling the ecosystem structure of a headwater stream by strongly influencing the physical and chemical parameters of the water, a finding also determined by other studies (Biggs et al. 2005, Sabater and Tockner 2009, Dalu et al. 2017). The modulation of water flow by factors such as seasonality, damming, and the precipitation regime had different effects on the ecosystem structure of the stream.

Ecosystem structure of headwater streams is driven by seasonality

The ecological structure of this headwater stream followed a marked seasonal pattern driven by the water flow and temperature. Winter was the most differentiated season because the cold temperatures and the low water flow due to water retention in snow allowed the bloom of the macroalga *Hydrurus foetidus*, a rheophilic and psychrophilic chrysophyte algae that generates long filaments where many diatoms grow and macroinvertebrates thrive (Klaveness 2019). The winter bloom of this macroalga increased the algal production-related variables because of its macroscopic structure, but also the algal genera richness because its thalli structure supports diatom growth. This primary production increase provided a valuable food resource for emerging waterborne insect larvae (Milner et al. 2009), especially for chironomids (Klaveness 2019), which promoted the winter peak of macroinvertebrate biomass and diversity. This winter production and biodiversity increase is key to maintaining the ecosystem structure of headwater streams with similar characteristics to the Catllar stream.

Antagonistic effects of damming and metal and metalloid pollution on the ecosystem structure of headwater streams

The presence of the dam caused downstream water flow reduction, with clear differences in physical and chemical parameters of the water from the upstream section of the stream. Despite this difference, the impacts of

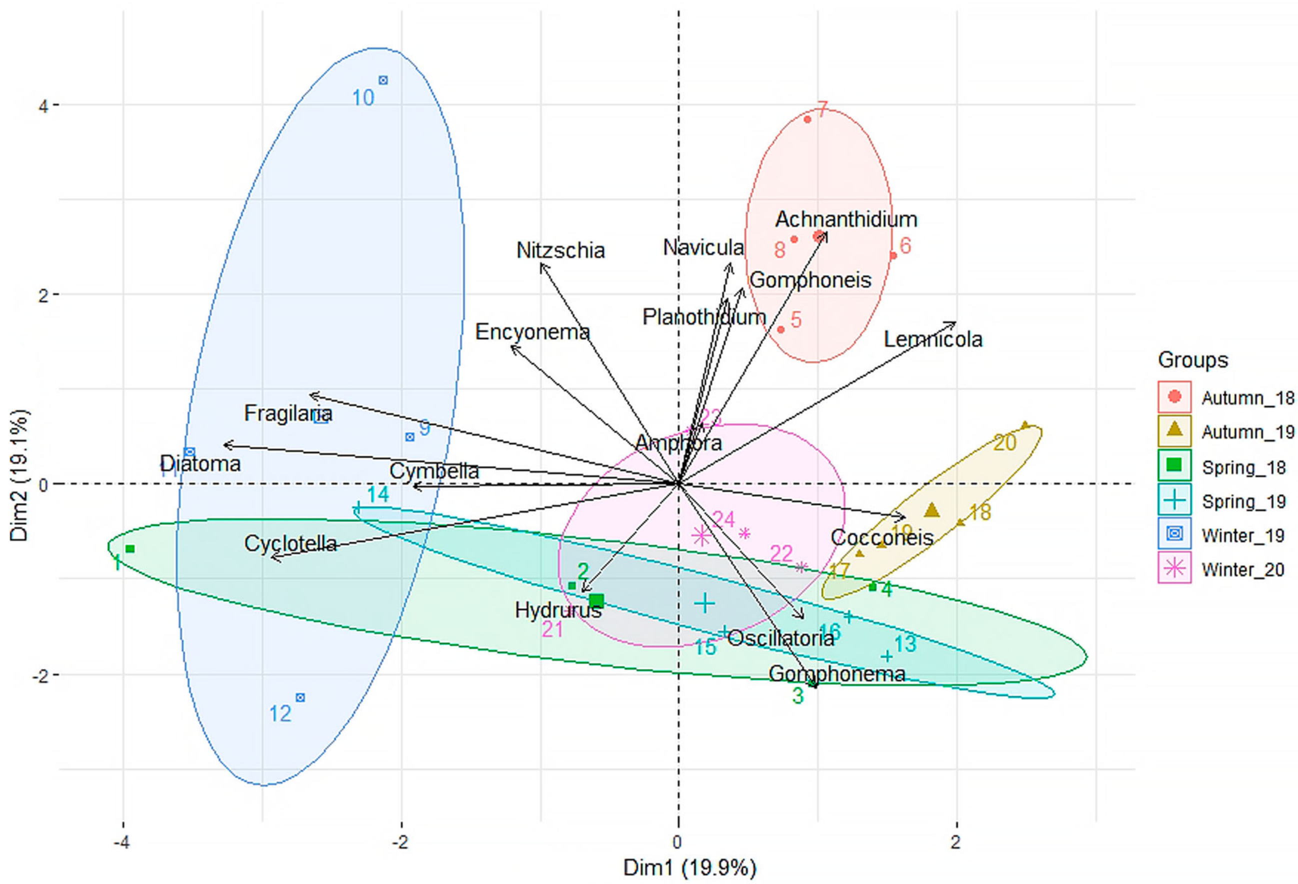

Figure 3. PCA plot showing community differences between seasons for algal genera (expressed in biovolume) found in biofilms. The macroalga *Hydrurus foetidus* was found in the streambed during cold seasons.

damming were weakly reflected on the ecosystem structure of the stream, a finding also observed by Almeida et al. (2013), because macroinvertebrates are resilient to flow regulations. The water flow reduction and sediment retention caused by the dam increased the organic matter proportion of the biofilms (Sabater and Tockner 2009), making them more digestible and attractive as a food resource for the macroinvertebrate community (Ceola et al. 2013), which promoted the macroinvertebrate biomass increase downstream of the dam. The water flow reduction caused by damming also had an indirect impact on the ecosystem structure of the stream through its interaction with the metal and metalloid pollution. Downstream of the dam, metal and metalloid concentrations were much lower despite the flow reduction because the particular disposition of the dam diverted the upstream water and caused most of the downstream water to come from an adjacent stream not impacted by metal pollution. This situation was reflected in the ecosystem structure with a downstream increase in the EPT index, macroinvertebrate groups sensitive to metal pollution (De Jonge et al. 2008) that can be replaced by others such as Chironomidae in polluted streams (Li et al. 2010). The diversion of polluted water along with the organic matter content increase in the biofilms promoted the increase in downstream macroinvertebrate biomass and diversity; consequently, damming and pollution had an antagonistic effect on the ecosystem structure of the stream. Direct impacts of damming might not be strongly reflected on the ecosystem structure of headwater streams, but the water flow changes from a dam could alter the physical and chemical characteristics of the downstream water; therefore, indirect impacts on the ecosystem structure should be considered when assessing how damming affects headwater streams.

Synergic effects of seasonality with a precipitation decrease and damming on the ecosystem structure of headwater streams

During low water availability, water flow of the stream decreased and its conductivity increased, which occurred during dry precipitation regimes and in winter because of water retention in snow. The interactive effects of a dry precipitation regime and the water

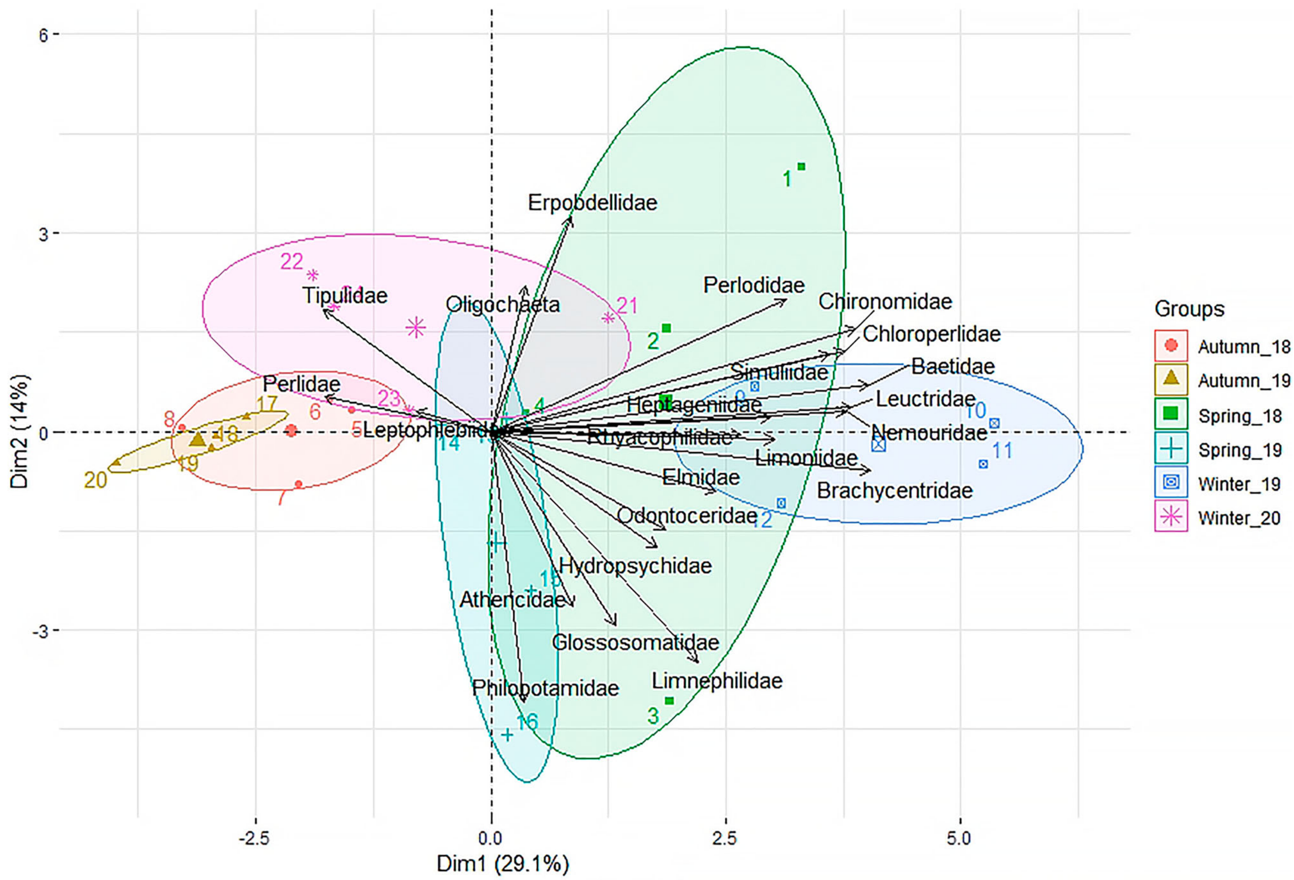

Figure 4. PCA plot showing community differences between seasons for macroinvertebrate families (expressed in biomass).

retention in snow during winter had a synergistic effect on the ecosystem structure of the stream by greatly reducing the water flow and increasing its temperature. This combination caused an increase in biofilm and algal biomass as well as macroinvertebrate biomass and abundance, but also a decrease in algal and macroinvertebrate diversity. These results suggest that the algal biomass increase did not support a better-quality food resource for macroinvertebrates, causing a loss in diversity and ecological quality through mechanisms similar to eutrophication. The low flow conditions of the stream increased the water temperature, light penetration, and nutrient concentrations in the water, promoting a shift to an autotrophic state and increasing

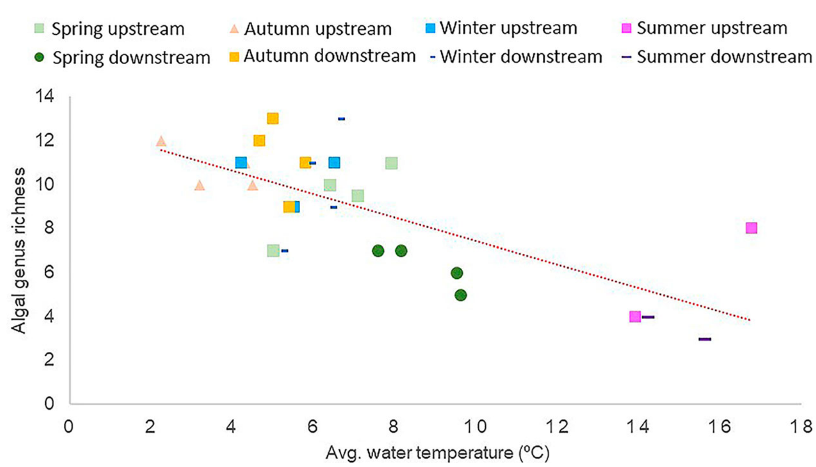

Figure 5. Algal genera richness in biofilms versus average water temperature of the stream for seasons and relative position from the dam (upstream and downstream).

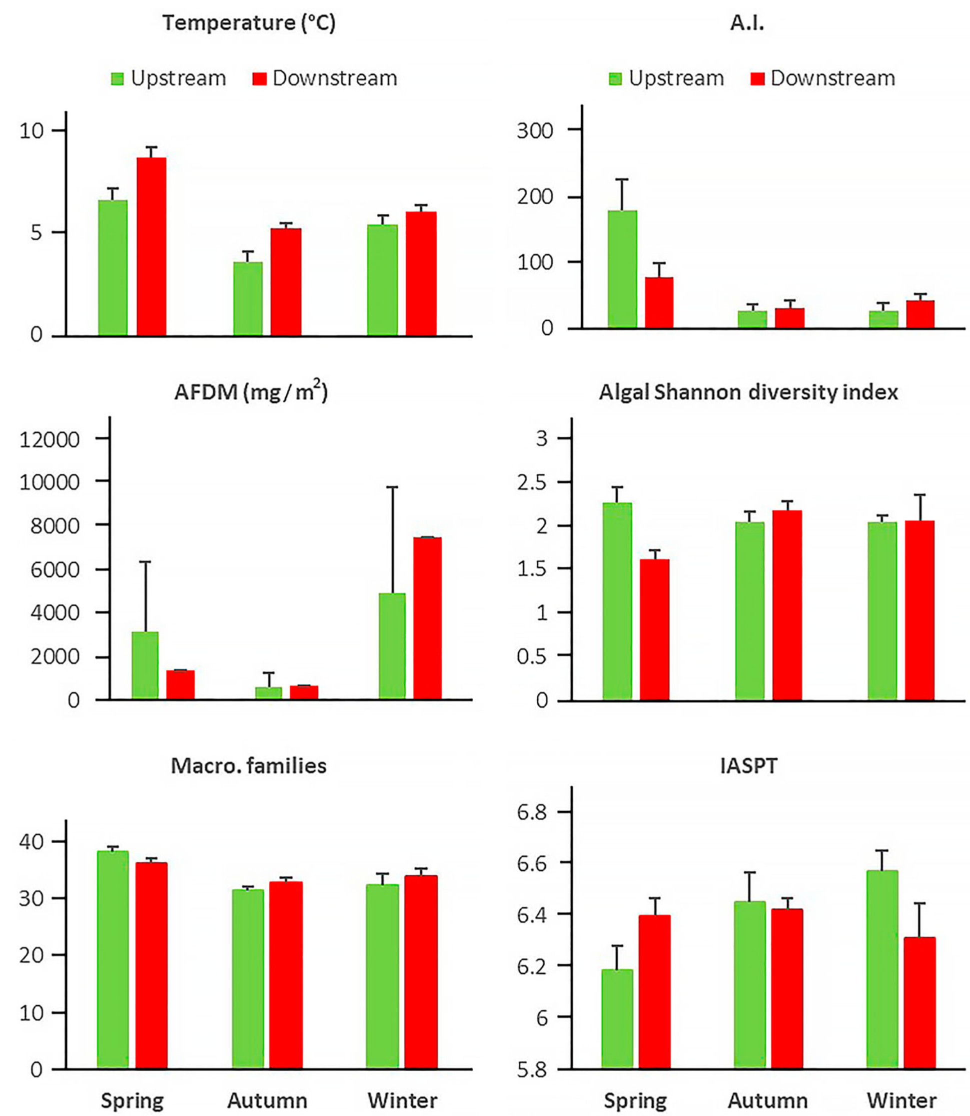

Figure 6. Effects of position from the dam for each season on the average values (with standard error) of water temperature, biofilm autotrophic index, biofilm ash free dry mass (AFDM), algal Shannon diversity in biofilm, macroinvertebrate (Macro.) number of families, and the Iberian Average Score per Taxon (IASPT) quality index. The effects of position on these variables differ significantly between seasons (Table 2).

the benthic biofilm biomass (Hilton et al. 2006, Yang et al. 2008, Sabater and Tockner 2009, Dodds and Smith 2016). The ecosystem structure of headwater streams requires a stable seasonal pattern of a cold, slow water flow in winter and a faster flow in spring from snowmelt. This biodiversity loss could be promoted by climate change through a warming of stream water and a decrease in precipitation. Climatic models predict this result in the Pyrenees (López-Moreno et al. 2008), and similar cases could occur in headwater streams from other mountain ranges with similar characteristics. In this study, we observed that the warming water and biodiversity loss was also promoted by seasonality and the precipitation regime synergically interacting with damming during low water availability. Consequently, the impacts of climate change on the ecosystem structure of headwater streams could be exacerbated in those affected by dams and other structures that reduce water flow and increase its temperature.

Metal and metalloid pollution in headwater streams could be exacerbated in a climate change context

Most of the metals and metalloids found in the Catllar stream are commonly found in aquatic systems affected by wastewater from mining (Zhou et al. 2008, Guasch et al. 2009) and are known to cause structural changes

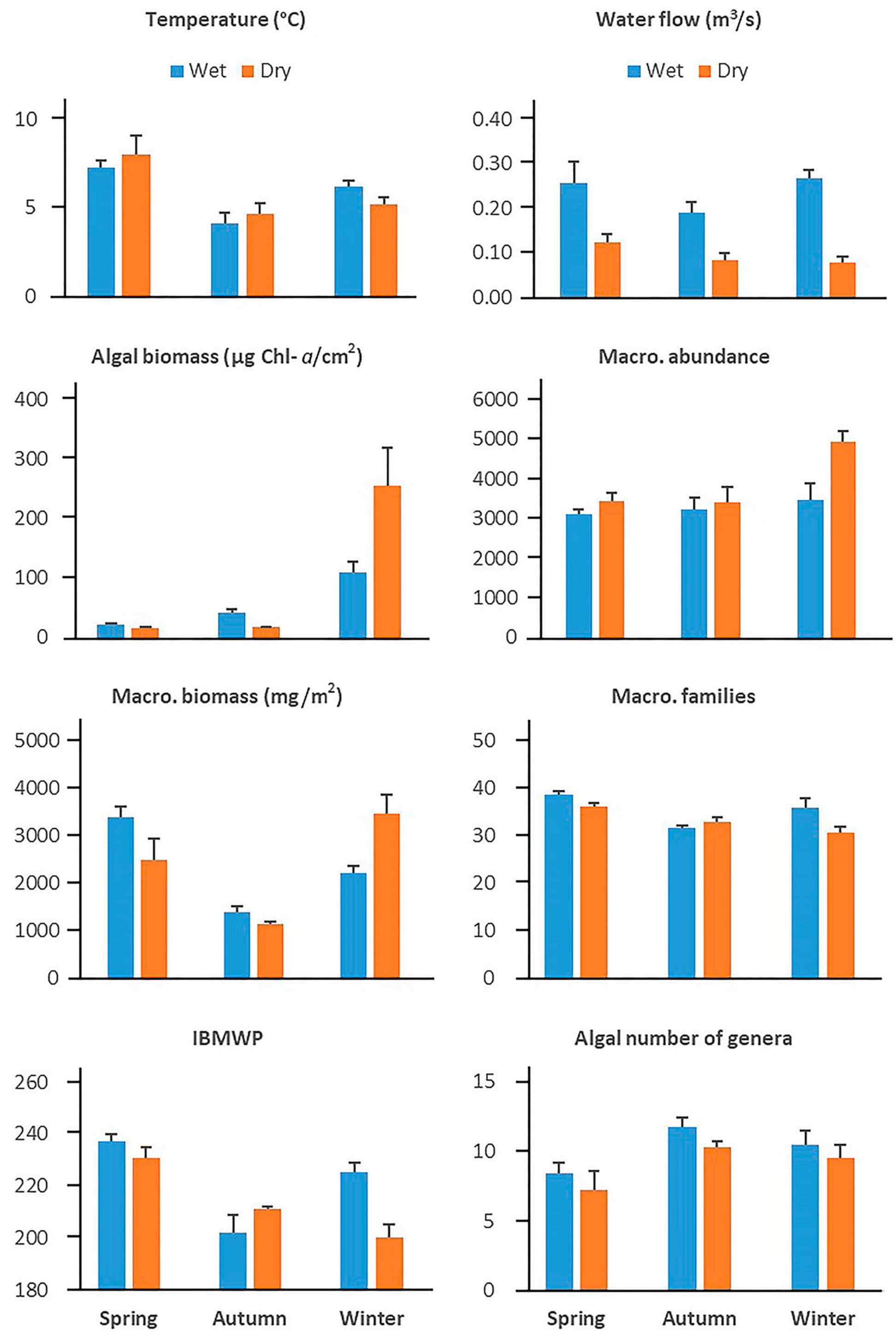

Figure 7. Effects of precipitation regime for each season on the average values (with standard error) of water temperature, water flow, algal biomass, algal number of genera, macroinvertebrate biomass and abundance, macroinvertebrate (Macro.) number of families, and the Iberian Biological Monitoring Working Party (IBMWP) quality index. Note the effects of the precipitation regime on these variables differ significantly between seasons (see Table 2).

in biofilms (Lawrence et al. 2004). Algal production in biofilms declines with high As (Tuulaikhuu et al. 2015, Barral-Fraga et al. 2020) and Ni concentrations (Lawrence et al. 2004), among other metals (Zhou et al. 2008), and the As concentration in the water column and in biofilms in this stream was higher during the low precipitation regime because of the low water availability. The stream had a lower water flow that was warmer, more acidic, and had a higher conductivity, factors that promote the availability of metals and

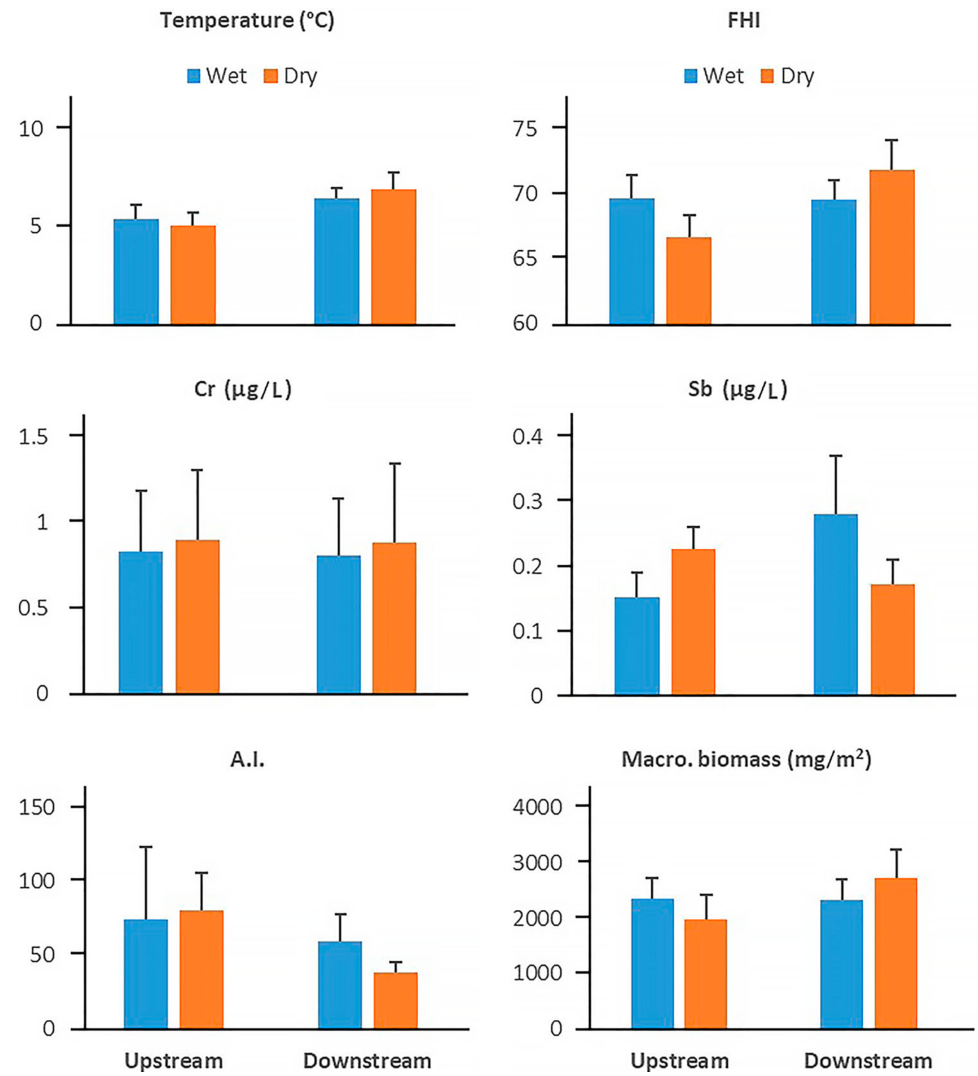

Figure 8. Effects of precipitation regime for each position on the average values (with standard error) of water temperature, fluvial habitat index (FHI), chromium (Cr) and antimony (Sb) concentrations in water, biofilm autotrophic index (A.I.), and macroinvertebrate (Macro.) biomass. Notice that the effects of the precipitation regime on these variables differ significantly between positions (Table 2 and 4).

metalloids in the water column (Kar et al. 2008, Li et al. 2013). Climate change is expected to decrease precipitation and increase water temperature in the Pyrenees (López-Moreno et al. 2008) as well as in other mountain ranges (Jones and Rinehart 2010, Bogan et al. 2014, Nukazawa et al. 2018), which could aggravate the impacts on headwater streams affected by mining by increasing metal concentrations in biofilms and uptake of metals into higher trophic levels (Gümgüm et al. 1994, Barral-Fraga et al. 2020).

Recommendations for the conservation of headwater streams

Conservation of headwater streams is a fundamental consideration that must be addressed to effectively preserve freshwater ecosystems (Biggs et al. 2017). Because up to 90% of a river's flow is derived from headwater streams (Saunders et al. 2009), they play a vital role in whole river basins and are fundamental for the correct maintenance of the ecological integrity of a river network. In a climate change context, these ecosystems play a key role in river basins by providing water, ameliorating floods, transferring nutrients and carbon, intercepting pollutants, and supplying sediments (Biggs et al. 2017). Considering that the presence of dams is common in headwater streams from many mountain ranges and that climate change will decrease the water availability in these ecosystems, the maintenance and restoration of a sufficient ecological flow in dam-impounded streams should be promoted (Rudra 2018). More research is needed on the resistance and resilience of small headwater stream ecosystems to anthropogenic disturbances (Biggs et al. 2017), and our results highlight the importance of field studies in the assessment of how multiple anthropogenic stressors interact in an applied context.

Conclusions

This case study showed the role of seasonality and the effects of both damming and a reduction in precipitation on the ecosystem structure of a headwater stream. These stressors, by themselves, negatively impacted the biofilm and macroinvertebrate communities of the stream. They caused water warming and flow reduction that promoted stream autotrophy, increasing the algal and macroinvertebrate biomass but causing biodiversity and ecological quality loss in a process similar to eutrophication. Additionally, both stressors had a synergic interaction with water retention in snow that occurs during the cold season by exacerbating those impacts, threatening the high levels of production and biodiversity in winter that sustain a functional ecosystem structure for headwater streams. Many streams are affected by metal and metalloid pollution, and our case study demonstrated that their ecological integrity is especially threatened by water flow reduction during cold seasons and low precipitation regimes that can increase the concentration of metals and metalloids in the water column and biofilms. Despite this, damming can also diminish the impact of pollution by diverting polluted water, thus increasing downstream water quality. In summary, the maintenance of a stable ecosystem structure in headwater streams can be threatened by damming in the current climate change context, with an expected water flow decrease and temperature increase in many mountain ranges, especially in headwater streams simultaneously affected by metal and metalloid pollution.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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